

Final Shipboard Report According to International Maritime Organization Guideline 8

Trojan Marinex™ BWT 250

Golden Bear Facility
Vallejo, California

Final Shipboard Report According to International Maritime Organization Guideline 8

Trojan Marinex™ BWT 250

Golden Bear Facility
Vallejo, California

Facility
Director:

William T. Davidson
Golden Bear Facility

Lead
Scientist

Nicolas Welschmeyer, PhD
Golden Bear Facility / Moss Landing
Marine Laboratories

Quality Officer:

Richard Muller
Golden Bear Facility

Date: 12-December-2013

Rev. No.: Rev. 1



CAL MARITIME
GOLDEN BEAR FACILITY

Table of Contents

1	EXECUTIVE SUMMARY	1
2	INTRODUCTION	6
3	GOLDEN BEAR FACILITY DESCRIPTION	7
4	BALLAST WATER MANAGEMENT SYSTEM DESCRIPTION	10
4.1	Ballasting Operation	10
4.2	Deballasting Operation	11
5	SHIPBOARD TESTING EXECUTION AND LOGISTICS	12
5.1	Shipboard Test Process	12
5.2	Sampling Method	17
5.3	Sample Identification and Labeling	19
6	SUMMARY OF ANALYTICAL METHODS	20
6.1	Biological Efficacy	20
6.2	Water Quality	22
7	MODIFICATIONS TO TEST METHODS AND SAMPLING PROCEDURES	24
7.1	Project Plan Execution	24
7.2	Shipboard Test 1	25
7.3	Shipboard Test 2	26
7.4	Shipboard Test 3	26
7.5	Shipboard Test 4	27
8	RESULTS	28
8.1	Biological Efficacy	28
8.2	Water Quality	46
9	DISCUSSION AND CONCLUSION	50
10	ACKNOWLEDGEMENTS	53

List of Tables

Table 1 - Summary of Shipboard Biological Efficacy Results	2
Table 2 - Summary of Shipboard Engineering Parameters and Water Quality Data	4
Table 3 - Shipboard Test Information	13
Table 4 - Challenge Water Sample Parameters and Processing Methods	23
Table 5 - Organisms $\geq 50 \mu\text{m}$ Results	30
Table 6 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, MPN Results	35
Table 7 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, Flow Cytometry Results	37
Table 8 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, C-14 Primary Production Results	38
Table 9 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, Variable	39
Table 10 - Organisms $\geq 10 \mu\text{m}$, Adenosine Triphosphate (ATP)	41
Table 11 - Indicator Microbes $< 10 \mu\text{m}$, <i>E. coli</i> Results	43
Table 12 - Indicator Microbes $< 10 \mu\text{m}$, <i>Enterococci</i> Results	44
Table 13 - Indicator Microbes $< 10 \mu\text{m}$, Heterotrophic Plate Count	45
Table 14 - Continuous In-Situ and In-line Water Quality Results	46
Table 15 - POC Results	47
Table 16 - DOC Results	47
Table 17 - TSS Results	48
Table 18 - Chlorophyll α Results	48
Table 19 - pH Results	49
Table 20 - UV Transmittance Results	49

List of Figures

Figure 1 - Training Ship Golden Bear, Vallejo, California	7
Figure 2 - Ballasting and Deballasting Process Flow	10
Figure 3 - Test 1 Pressure Measurement Across BWMS Filter	15
Figure 4 - Test 2 Pressure Measurement Across BWMS Filter	15
Figure 5 - Test 3 Pressure Measurement Across BWMS Filter	16
Figure 6 - Test 4 Pressure Measurement Across BWMS Filter	16
Figure 7 - Typical Pitot Sample Port Connection and Pitot Tube Detail	18
Figure 8 - Zooplankton and Microbe Sample Station	19
Figure 9 - Common $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ Phytoplankton and $\geq 50 \mu\text{m}$ Zooplankton Observed	29
Figure 10 - Organisms $\geq 50 \mu\text{m}$ Results Comparison	30
Figure 11 - Phytoplankton Observed in MPN Grow-out Experiments	34
Figure 12 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, MPN Results Comparison	35
Figure 13 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, MPN Uptake and Treatment Discharge Results	36
Figure 14 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, Flow Cytometry Results Comparison	37
Figure 15 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, C-14 Primary Production Results Comparison	38

Figure 16 - Variable Chlorophyll-fluorescence (PAM) Results Comparison _____	39
Figure 17 - Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$, Variable Chlorophyll-fluorescence (PAM) Light Curves _____	40
Figure 18 - Organisms $\geq 10 \mu\text{m}$, Adenosine Triphosphate (ATP) Results Comparison _____	41
Figure 19 - Indicator Microbes $< 10 \mu\text{m}$, <i>E. coli</i> Results Comparison _____	43
Figure 20 - Indicator Microbes $< 10 \mu\text{m}$, <i>Enterococci</i> Results Comparison _____	44
Figure 21 - Indicator Microbes $< 10 \mu\text{m}$, Heterotrophic Plate Count Results Comparison _____	45

Appendices

Appendix A _____	Test Quality Assurance Plan Red-line
Appendix B _____	BWMS Maintenance Log
Appendix C _____	Project Training Records
Appendix D _____	Ballasting Operations Log
Appendix E _____	GBF Automation System Outputs
Appendix F _____	Raw Test Data
Appendix G _____	Data Quality Indicators
Appendix H _____	Organism Composition and Taxonomy

References

Cochran, W.G. 1950. Estimation of bacterial densities by means of “most probable number”. *Biometrics* 6: 105-116.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 281(5374):237–240.

Fujieki, Lance. “Primary Production Time-series”. *Hawaii Ocean Time-series Data Organization & Graphical System (HOT-DOGS®)*. (n.d). Web. 1 March 2012. <<http://hahana.soest.hawaii.edu/hot/hot-dogs/ppseries.html>>.

Gollasch, S., Rosenthal, H., Botnen, H., Hamer, J. and others. 2000. Fluctuations of zooplankton taxa in ballast water during short-term and long-term ocean-going voyages. *Int Rev Hydrobiol* 85:597–608.

Generic Protocol for the Verification of Ballast Water Treatment Technologies (ETV Protocol), US Environmental Protection Agency, Environmental Technology Verification Program. September 2010.

Guidelines for Approval of Ballast Water Management Systems (G8), Annex 4, Resolution MEPC.174(58), Marine Environment Protection Committee, 10 October 2008.

Guidelines for Ballast Water Sampling (G2), Annex 3, Resolution MEPC.173(58), Marine Environment Protection Committee, 10 October 2008.

IMO, International Convention for the Control and Management of Ships’ Ballast

Water and Sediments, 2004.

Karl, D. M. 1993. Total microbial biomass estimation derived from the measurement of particulate adenosine-5'-triphosphate. In P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole (eds.), *Current methods in aquatic microbial ecology*, pp. 359-368. Lewis Publishers, Boca Raton, Florida.

Maxwell, K and G.N. Johnson. 2000. Chlorophyll fluorescence – A practical guide. *J. Exp. Bot.* 51:659-668.

Miller, A.W., M. Frazier, G.E. Smith, E.S. Perry, G.M. Ruiz and M. Tamburri. 2011. Enumerating Sparse Organisms in Ships' Ballast Water: Why Counting to 10 Is Not So Easy. *Environ Sci Technol.* 45(8): 3539–3546

Steinberg, M.K., E.J. Lemieux, and L.A. Drake. 2011. Determining the viability of marine protists using a combination of vital, fluorescent stains. *Marine Biology* 158: 1–7.

Woomer, P., J. Bennett, and R. Yost. 1990. Overcoming the inflexibility of most-probable-number procedures. *Agron. J.* 82: 349-353.

List of Abbreviations

<u>Abbreviation</u>	<u>Description</u>
ATP	Adenosine Triphosphate
BWMS	Ballast Water Management System
chl α	Chlorophyll α
CFU	Colony Forming Units
CHN	Carbon-Hydrogen-Nitrogen
CMA	California Maritime Academy
CSU	California State University
$^{\circ}\text{C}$	Degree Centigrade
C/L	Carbon per Liter
CV	Coefficient of Variation
DC	Direct Current
DIC	Dissolved Inorganic Carbonate
DNA	Deoxyribonucleic Acid
DNV	Det Norske Veritas
DOC	Dissolved Organic Carbon
DPM/gC	Disintegrations per Minute per Gram of Carbon
ESD	Equivalent Spherical Diameter
FDA	Forward Scatter
FSC	Fluorescein Diacetate
ft ²	Square Feet
Fv/Fm	Variable Fluorescence / Maximum Fluorescence
G2	IMO Guidelines for Ballast Water Sampling
G8	IMO Guidelines for Approval of Ballast Water Management Systems
GBF	Golden Bear Facility
HPC	Heterotrophic Plate Count
hr	Hour
IMO	International Maritime Organization
kW	Kilowatts
L	Liter
m	Meter
m ³	Cubic Meters
m ³ /hr	Cubic Meters per Hour
MARAD	United States Maritime Administration
mA	Milliamp
mg	Milligram
MLML	Moss Landing Marine Laboratory
MPN	Most Probable Number

<u>Abbreviation</u>	<u>Description</u>
mM	MilliMoles
n	Sample Number
n/a	Not Analyzed
ng	Nanogram
nm	Nanometer
n/r	Not Required
PAM	Pulse Amplitude Modulated
pg	Picogram
POC	Particulate Organic Carbon
PON	Particulate Organic Nitrogen
ppm	Parts per Million
QAPP	Quality Assurance Project Plan
QMP	Quality Management Plan
S.D.	Standard Deviation
SOP	Standard Operating Procedure
TQAP	Test Quality Assurance Plan
TSS	Total Suspended Solids
μCi	Microcurie
μL	Microliters
μm	Micrometers (Microns)
μMole	Micromole
USEPA	United States Environmental Protection Agency
USTS	United States Training Ship
UV	Ultra-violet

1 EXECUTIVE SUMMARY

The Golden Bear Facility (GBF) conducted a series of four shipboard verification tests on the United States Training Ship (USTS) *Golden Bear* using a ballast water management system (BWMS) furnished by Trojan Marinex. The BWMS utilized a combination of mechanical separation (filtration) and ultra-violet (UV) radiation to achieve removal/inactivation of ambient organisms. In all shipboard tests, the Trojan Marinex™ BWT 250 met or exceeded the International Maritime Organization (IMO) D-2 discharge standards specified in the *International Convention for the Control and Management of Ships' Ballast Water and Sediments* (2004). All shipboard tests were conducted in accordance with IMO *Guidelines for Approval of Ballast Water Management Systems* (G8) (Resolution MEPC.174(58)) and in consultation with the type approval administration, Det Norske Veritas (DNV).

GBF preparation and planning efforts were conducted from January through March of 2012 and resulted in acceptance of a project-specific Test Quality Assurance Plan (TQAP) by DNV (see Appendix A). During this time, testing efforts were witnessed by DNV in order to ensure adherence to GBF quality control and quality assurance procedures.

The shipboard biological efficacy tests were conducted with the Trojan Marinex™ BWT 250 from March 2012 through March 2013 following the procedures specified in the TQAP. The shipboard test series met the guidelines in IMO G8 for shipboard testing, though it deviated from the approved TQAP and test plan in the following instances:

- The shipboard Quality Assurance Project Plan (QAPP) and Standard Operating Procedures (SOPs) specified four biological efficacy tests with uptake in four distinct locations representing unique geographic locations, water chemistry, and biological assemblages. However, at the BWMS manufacturer's request, test uptake locations were moved or cancelled in Test 2, 3 and 4 resulting in all four test uptakes occurring in Oakland, California (central San Francisco Bay).
- The TQAP included test plans for combined shipboard and land-based testing; however, land-based testing was not completed.

Shipboard Test 1 consisted of three specific ballasting events: a combined uptake event to sample both treatment and control ballast water followed by treatment discharge and control discharge. As requested by DNV, the test plan was revised such that Tests 2, 3, and 4 consisted of four specific ballasting events: treatment uptake, control uptake, treatment discharge, and control discharge. During each uptake operation, challenge water was taken up from the local waters and pumped into treatment and control ballast tanks in the USTS *Golden Bear* according to flow rates and volumes specified in the TQAP. The vessel travelled to a new location during the ballast water holding time of five days. Upon discharge, the treated ballast water was processed by the BWMS again and directed overboard. In Tests 1, 2, and 4, treatment on discharge did not include filtration and relied on UV only. Test 3 utilized UV and filtration on uptake and discharge. Control ballast water was discharged without treatment. During all ballast events, the ballast water was

sampled and various chemical, biological, and engineering parameters were monitored (see Section 8).

Tables 1 and 2 below provide a summary of biological efficacy results, engineering parameters and water quality results relevant to all shipboard tests. This information includes valid IMO G8 test criteria, mean observed values of live organisms, and standard deviations. In all four shipboard tests, water quality met minimum uptake criteria and the biological efficacy results indicate satisfactory achievement of all IMO D-2 standards. All corroborative assays conducted in addition to required IMO required analyses support the findings above with the exception of cell-specific FDA viable tagging (flow cytometry) which appear to indicate false positive scores (false identification of viable cells) for UV treated discharge samples.

Table 1 - Summary of Shipboard Biological Efficacy Results

Parameters			IMO G8 Criteria	Test 1 ^a	Test 2	Test 3	Test 4
Treatment & Control Uptake Location (Latitude / Longitude)				37° 47.195' N / 122° 23.045' W	37° 50.6' N / 122° 26.4' W	37° 49.195' N / 122° 23.045' W	37° 49.195' N / 122° 23.045' W
Treatment Uptake Date (dd-mm-yy)				17-Mar-12	1-May-12	7-Oct-12	20-Mar-13
≥50 µm (organisms/m ³)	Mean (S.D.)	≥ 100		66,513 (11,967)	82,849 (16,327)	117,796 (28,267)	47,659 (8,129)
<50 µm & ≥10 µm, MPN (organisms/mL)	Mean (S.D.)	≥ 100		320 (154)	1,233 (153)	480	100
<50 µm & ≥10 µm, Flow Cytometry ^b (organisms/mL)	Mean (S.D.)	≥ 100		624 (108)	433 (59)	469 (85)	1,330 (113)
<50 µm & ≥10 µm, C-14 Primary Production ^b (µg C/(L·day))	Mean (S.D.)	n/r		215.27 (3.29)	615.51 (95.36)	75.06 (4.95)	27.20 (3.05)
<50 µm & ≥10 µm, Variable Fluorescence ^b (PAM) (Fv/Fm)	Mean (S.D.)	n/r		0.664 (0.034)	0.708 (0.018)	0.670 (0.037)	0.639 (0.006)
<50 µm & ≥10 µm, ATP ^b (ng/L)	Mean (S.D.)	n/r		n/a	n/a	110.59 (14.61)	158.99 (34.38)
Indicator Microbes, <i>E. coli</i> (CFU/100 mL)	Mean (S.D.)	n/r		0.22 (0.44)	n/a	1.00 (1.00)	0.67 (0.58)
Indicator Microbes, <i>Enterococci</i> (CFU/100 mL)	Mean (S.D.)	n/r		181.40 (31.30)	n/a	98.67 (26.96)	200.50 (0.00)
Indicator Microbes, <i>Vibrio cholerae</i> (01/0139) (CFU/100 mL)	Mean (S.D.)	n/r		0.00	0.00	0.00	0.00
Indicator Microbes, Heterotrophic Plate Counts ^b (CFU/mL)	Mean (S.D.)	n/r		1,580 (550)	917 (535)	420 (192)	1,067 (188)
Control Uptake Date (dd-mm-yy)				17-Mar-12	1-May-12	7-Oct-12	20-Mar-13
≥50 µm (organisms/m ³)	Mean (S.D.)	≥ 100		66,513 (11,967)	50,232 (6,660)	98,020 (17,249)	37,340 (5,458)
<50 µm & ≥10 µm, MPN (organisms/mL)	Mean (S.D.)	≥ 100		320 (154)	2,000 (600)	630.00	170
<50 µm & ≥10 µm, Flow Cytometry ^b (organisms/mL)	Mean (S.D.)			624 (108)	188 (31)	450 (81)	1,000 (180)
<50 µm & ≥10 µm, C-14 Primary Production ^b (µg C/(L·day))	Mean (S.D.)	n/r		215.27 (3.29)	396.32 (38.76)	63.58 (11.93)	28.94 (2.07)
<50 µm & ≥10 µm, Variable Fluorescence ^b (PAM) (Fv/Fm)	Mean (S.D.)	n/r		n/a	0.687 (0.018)	0.715 (0.057)	0.593 (0.081)
<50 µm & ≥10 µm, ATP ^b (ng/L)	Mean (S.D.)	n/r		n/a	n/a	105.77 (24.91)	42.72 (19.14)
Indicator Microbes, <i>E. coli</i> (CFU/100 mL)	Mean (S.D.)	n/r		0.22 (0.44)	0.00 (0.00)	3.50 (2.72)	2.37 (0.64)
Indicator Microbes, <i>Enterococci</i> (CFU/100 mL)	Mean (S.D.)	n/r		181.40 (31.30)	86.07 (33.37)	134.73 (36.35)	146.47 (47.93)
Indicator Microbes, <i>Vibrio cholerae</i> (01/0139) (CFU/100 mL)	Mean (S.D.)	n/r		0.00	0.00	0.00	0.00

Parameters		IMO G8 Criteria	Test 1 ^a	Test 2	Test 3	Test 4
Indicator Microbes, Heterotrophic Plate Counts ^b (CFU/mL)	Mean (S.D.)	n/r	1,580 (550)	210 (75)	247 (145)	1,830 (339)
Treatment & Control Discharge Location (Latitude / Longitude)			38° 03.8' N / 122° 13.9' W	33° 22.4' N / 118° 16.5' W	38° 03.8' N / 122° 13.9' W	38° 03.8' N / 122° 13.9' W
Treatment Discharge Date (dd-mm-yy)			22-Mar-12	6-May-12	12-Oct-12	25-Mar-13
≥50 µm (organisms/m ³)	Mean (S.D.)	<10	1.60 (0.35)	0.00 (0.00)	0.10 (0.17)	2.05 (0.35)
<50 µm & ≥10 µm, MPN (organisms/mL)	Mean (S.D.)	<10	0.055 (0.003)	0.058 (0.003)	<0.056	0.056 (0.00)
<50 µm & ≥10 µm, Flow Cytometry ^b (organisms/mL)	Mean (S.D.)	<10	15 (16)	3 (5)	17 (17)	35 (18)
<50 µm & ≥10 µm, C-14 Primary Production ^b (µg C/(L·day))	Mean (S.D.)	n/r	1.31 (0.24)	0.93 (0.18)	0.35 (0.04)	4.76 (0.69)
<50 µm & ≥10 µm, Variable Fluorescence ^b (PAM) (Fv/Fm)	Mean (S.D.)	n/r	0.035 (0.039)	0.045 (0.077)	0.165 (0.073)	0.108 (0.097)
<50 µm & ≥10 µm, ATP ^b (ng/L)	Mean (S.D.)	n/r	n/a	n/a	0.07 (0.04)	1.52 (0.30)
Indicator Microbes, <i>E. coli</i> (CFU/100 mL)	Mean (S.D.)	<250	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Indicator Microbes, <i>Enterococci</i> (CFU/100 mL)	Mean (S.D.)	<100	0.13 (0.35)	0.00 (0.00)	0.33 (0.58)	1.00 (1.00)
Indicator Microbes, <i>Vibrio cholerae</i> (01/0139) (CFU/100 mL)	Mean (S.D.)	<1	0.00	0.00	0.00	0.00
Indicator Microbes, Heterotrophic Plate Counts ^b (CFU/mL)	Mean (S.D.)	n/r	207 (145)	83 (61)	120 (26)	147 (45)
Control Discharge Date (dd-mm-yy)			22-Mar-12	6-May-12	12-Oct-12	25-Mar-13
≥50 µm (organisms/m ³)	Mean (S.D.)	>10	32,168 (4,036)	49,290 (6,009)	45,322 (5,013)	22,826 (2,492)
<50 µm & ≥10 µm, MPN (organisms/mL)	Mean (S.D.)	>10	57 (89)	367 (237)	92 (121)	12 (9)
<50 µm & ≥10 µm, Flow Cytometry ^b (organisms/mL)	Mean (S.D.)	>10	114 (15)	131 (30)	92 (26)	306 (53)
<50 µm & ≥10 µm, C-14 Primary Production ^b (µg C/(L·day))	Mean (S.D.)	n/r	45.48 (11.59)	112.29 (8.22)	10.44 (2.98)	14.27 (1.10)
<50 µm & ≥10 µm, Variable Fluorescence ^b (PAM) (Fv/Fm)	Mean (S.D.)	n/r	0.442 (0.108)	0.729 (0.019)	0.521 (0.094)	0.436 (0.054)
<50 µm & ≥10 µm, ATP ^b (ng/L)	Mean (S.D.)	n/r	n/a	n/a	18.19 (10.85)	21.17 (14.39)
Indicator Microbes, <i>E. coli</i> (CFU/100 mL)	Mean (S.D.)	n/r	0.00 (0.00)	0.33 (0.58)	16.03 (4.25)	0.33 (0.58)
Indicator Microbes, <i>Enterococci</i> (CFU/100 mL)	Mean (S.D.)	n/r	109.41 (29.19)	16.77 (7.90)	83.60 (8.49)	200.50 (0.00)
Indicator Microbes, <i>Vibrio cholerae</i> (01/0139) (CFU/100 mL)	Mean (S.D.)	n/r	0.00	0.00	0.00	0.00
Indicator Microbes, Heterotrophic Plate Counts ^b (CFU/mL)	Mean (S.D.)	n/r	2,820 (250)	1,033 (801)	960 (423)	963 (92)

a = Treatment and control uptake occurred sequentially and untreated water was sampled continuously as a combined event.

Data is duplicated in table for review purposes only.

b = Method performed as a corroborative analysis.

S.D. = standard deviation; n/r = not required; n/a = not analyzed

Table 2 - Summary of Shipboard Engineering Parameters and Water Quality Results

Parameters		Test 1 ^a	Test 2	Test 3	Test 4
Treatment & Control Uptake Location (Latitude / Longitude)		37° 47.195' N / 122° 23.045' W	37° 50.6' N / 122° 26.4' W	37° 49.195' N / 122° 23.045' W	37° 49.195' N / 122° 23.045' W
Treatment Uptake Date (dd-mm-yy)		17-Mar-12	1-May-12	7-Oct-12	20-Mar-13
BWMS Effluent Flow Rate (m ³ /hr)	Mean	231.8	242.7	205.3	250.0
Temperature (°C) (in-line)	Mean	13.0	13.8	18.0	14.5
Salinity (PSU) (in-situ)	Range	29.09-29.43	16.37-30.49	19.69-31.59	28.36-28.52
Dissolved Oxygen (mg/L)	Mean	8.97	8.89	7.85	8.64
POC (mg/L)	Mean (S.D.)	0.84 (0.02)	0.45 (0.02)	0.56	0.59 (0.16)
DOC (mg/L)	Mean (S.D.)	1.9 (0.06)	0.8 (0.15)	1.9	2.4
TSS (mg/L)	Mean (S.D.)	59.37 (1.69)	32.51 (3.35)	29.27 (1.65)	40.65 (2.22)
Chlorophyll α^b (µg/L)	Mean (S.D.)	2.26 (0.54)	9.21 (0.42)	3.18 (0.25)	5.38 (0.38)
pH	Mean (S.D.)	7.99 (0.01)	8.00 (0.01)	7.92 (0.01)	7.94 (0.01)
UV Transmittance ^b (%/cm)	Mean (S.D.)	78.78 (1.90)	90.71 (0.64)	87.50 (3.24)	93.04 (0.61)
UV Intensity ^c (mW/cm ²)	Mean	8.5	10.0	9.4	9.2
BWMS Energy Consumption (kW)	Mean	n/a ^d	12.68	12.66	12.67
Control Uptake Date (dd-mm-yy)		17-Mar-12	1-May-12	7-Oct-12	20-Mar-13
BWMS Effluent Flow Rate (m ³ /hr)	Mean	231.8	251.4	248.8	261.7
Temperature (°C) (in-line)	Mean	13.0	11.1	17.7	14.4
Salinity (PSU) (in-situ)	Range	29.09-29.43	27.47-33.13	31.8-31.8	26.90-28.37
Dissolved Oxygen (mg/L)	Mean	8.97	7.69	7.63	8.52
POC (mg/L)	Mean (S.D.)	0.84 (0.02)	0.48 (0.01)	0.45	1.56 (0.18)
DOC (mg/L)	Mean (S.D.)	1.9 (0.06)	1.5 (0.47)	2.2	2.4
TSS (mg/L)	Mean (S.D.)	59.37 (1.69)	26.14 (0.04)	23.14 (3.58)	105.37 (3.36)
Chlorophyll α^b (µg/L)	Mean (S.D.)	2.26 (0.54)	8.44 (1.11)	2.49 (0.28)	4.79 (0.33)
pH	Mean (S.D.)	7.99 (0.01)	7.89 (0.01)	7.94 (0.00)	7.95 (0.00)
UV Transmittance ^b (%/cm)	Mean (S.D.)	78.78 (1.90)	95.80 (1.41)	87.49 (3.24)	80.55 (3.78)
Treatment & Control Discharge Location (Latitude / Longitude)		38° 03.8' N / 122° 13.9' W	33° 22.4' N / 118° 16.5' W	38° 03.8' N / 122° 13.9' W	38° 03.8' N / 122° 13.9' W
Treatment Discharge Date (dd-mm-yy)		22-Mar-12	6-May-12	12-Oct-12	25-Mar-13
BWMS Effluent Flow Rate (m ³ /hr)	Mean	240.6	244.4	241.9	251.5
Temperature (°C) (in-line)	Mean	14.1	18.3	19.4	15.7
Salinity (PSU) (in-situ)	Range	28.7-29.34	29.40-29.59	n/a	27.31-28.32
Dissolved Oxygen (mg/L)	Mean	9.05	8.60	n/a	8.42
POC (mg/L)	Mean (S.D.)	0.37 (0.02)	0.47 (0.02)	0.46	0.30 (0.03)
DOC (mg/L)	Mean (S.D.)	n/a	n/a	n/a	n/a
TSS (mg/L)	Mean (S.D.)	47.71 (2.92)	14.68 (0.95)	27.37 (0.82)	39.40 (1.71)
Chlorophyll α^b (µg/L)	Mean (S.D.)	0.66 (0.12)	0.64 (0.03)	1.07 (0.11)	0.95 (0.15)
pH	Mean (S.D.)	7.92 (0.00)	8.00 (0.01)	7.91 (0.01)	7.93 (0.01)
UV Transmittance ^b (%/cm)	Mean (S.D.)	n/a	n/a	87.8 (1.54)	n/a
BWMS Energy Consumption (kW)	Mean	16.67	12.68	12.66	12.66

Parameters		Test 1 ^a	Test 2	Test 3	Test 4
Control Discharge Date (dd-mm-yy)		22-Mar-12	6-May-12	12-Oct-12	25-Mar-13
BWMS Effluent Flow Rate (m ³ /hr)	Mean	249.4	249.9	251.7	253.1
Temperature (°C) (in-line)	Mean	14.1	18.2	19.3	15.5
Salinity (PSU) (in-situ)	Range	26.61-27.14	30.42-32.84	n/a	25.77-25.94
Dissolved Oxygen (mg/L)	Mean	8.79	8.30	n/a	8.94
POC (mg/L)	Mean (S.D.)	0.26 (0.03)	0.55 (0.02)	0.39	0.59 (0.05)
DOC (mg/L)	Mean (S.D.)	n/a	n/a	n/a	n/a
TSS (mg/L)	Mean (S.D.)	40.47 (2.39)	17.40 (1.06)	24.31 (0.18)	42.68 (5.48)
Chlorophyll α^b (µg/L)	Mean (S.D.)	0.56 (0.15)	1.73 (0.14)	0.66 (0.04)	0.77 (0.09)
pH	Mean (S.D.)	7.93 (0.00)	7.94 (0.04)	7.88 (0.04)	7.88 (0.00)
UV Transmittance ^b (%/cm)	Mean (S.D.)	n/a	n/a	87.80 (1.54)	n/a

a = Treatment and control uptake occurred sequentially and untreated water was sampled continuously as a combined event. Data is duplicated in table for review purposes only.

b = Method performed as a corroborative analysis.

c = Means reported were onboard measurements by the BWMS.

d = One of three BWMS phase current transformers was not connected properly during the Test 1 uptake on 17-Mar-12. The mean BWMS energy consumption for this event was not representative of normal operations and is not reported for this event.

S.D. = standard deviation; n/r = not required; n/a = not analyzed

2 INTRODUCTION

The GBF objective was to determine if the Trojan Marinex™ BWT 250 complies the IMO D-2 discharge standard by conducting a series of shipboard tests onboard the USTS *Golden Bear*. Shipboard testing consisted of receiving, installing, and commissioning the Trojan Marinex™ BWT 250 in January and February 2012, and running a series of four tests between March 2012 and April 2013.

The documents below were developed to ensure quality control and quality assurance during testing. GBF's documentation control process is detailed in the Standard Operating Procedures (SOPs) of the Quality Management Plan (QMP).

The Test Quality Assurance Plan (TQAP) provided in Appendix A contains project-specific documentation, which includes the following:

- Project Plan (TQAP Volume I). This is a project-specific plan that identifies the established test objectives, test conditions, test facility, equipment evaluated, and test logistics including personnel and schedule.
- Quality Assurance Project Plans (QAPPs) (TQAP Volumes II and III). The land-based (Volume II) and shipboard (Volume III) QAPPs contain quality assurance and quality control measures for executing the SOPs to test the BWMS including operating parameters and data collection requirements. In addition, the protocols for evaluating biological and chemical conditions were established.
- Standard Operating Procedures (SOPs) (TQAP Volume IV). These procedures provided instruction for facility operations and ballast water sampling specific to this project.

The shipboard QAPP provides detailed descriptions of required inspections and commissioning procedures for the BWMS. The BWMS manufacturer was onsite for the installation, commissioning, and testing of the BWMS to provide confirmation of installation and readiness, and provide training for proper operation. This commissioning included a shakedown test of the BWMS, which stressed the BWMS so that any potential failure points would be apparent and could be remedied prior to initiating biological efficacy testing. All scheduled and unscheduled maintenance performed on the BWMS was noted on the BWMS Maintenance Log provided in Appendix B.

Water quality and biological analyses were performed at on-site and off-site laboratories in accordance to the SOPs. This report is the culmination of the analyses and reporting for all required data.

3 GOLDEN BEAR FACILITY DESCRIPTION

The USTS *Golden Bear* is a 500-foot ship owned by the United States Maritime Administration (MARAD) and operated by the California Maritime Academy (CMA) as part of the California State University (CSU) system. The ship is the primary asset of the GBF organizational structure. The vessel spends eight months per year docked in Vallejo, California taking occasional short trips in the San Francisco Bay area (see Figure 1). For the purposes of this report, “*Golden Bear*” refers to the ship, and “GBF” refers to program activities.

The *United States Naval Ship Maury* was transferred to the CMA in September of 1994 and renamed *Golden Bear*. Upon transfer, the vessel underwent modifications to adapt it to function in a training environment. As the vessel owner, MARAD provides maintenance and operational assistance to support CMA activities. Now based in Vallejo, California, *Golden Bear* provides a comprehensive training platform to those interested in working in the marine industry.

Length Overall	152.1 m
Molded Breadth	21.9 m
Molded Depth	12.8 m
Total Installed Power, Continuous	10,740 kW
Design Speed, Calm Water	20 knots
Design Deadweight	6974 tonnes
Ballast Flow Rate	90 to 435 m ³ /hr
Ballast Capacity	7141 m ³
Ballast Tanks	28



Figure 1 - Training Ship Golden Bear, Vallejo, California

The GBF program was developed in spring of 2010 to conduct research, development, testing, and evaluation of technologies and operational practices that serve to limit the impact of marine vessel operations on the environment. The *Golden Bear* has been outfitted to integrate a containerized BWMS with routine ship

ballasting operations. In addition, the *Golden Bear* has two dedicated ballast tanks, each with capacities greater than 435 m³, and associated piping outfitted to conduct controlled shipboard biological efficacy testing. For all shipboard tests referenced within this report, Tank 3-154-1 was used as the treatment tank and Tank 3-154-2 was used as the control tank.

The ship's pumping system can be varied between 90 m³/hr and more than 400 m³/hr and permits treatment on uptake and/or discharge. In this manner, the BWMS performs routine ballast water treatment under the stresses of normal ship operations during trans-oceanic voyages, and biological efficacy tests are performed under controlled conditions.

Specifically, the *Golden Bear* consists of:

- Dedicated onboard laboratory to enable rapid biological and chemical analyses to support GBF activities, including office space near the dock where biological samples are enumerated if necessary;
- Access to all ship's equipment; including the ballast water system, hull and apertures, bilge water and de-oiling equipment, sanitation system, and diesel engine exhaust systems; and
- Specialized equipment for GBF shipboard and campus facility purposes. To support BWMS evaluation, GBF installed a specialized pump and piping system, means of installing and integrating the BWMS, an automation system, and a ballast water sampling system.

The *Golden Bear* marine biology laboratory is located onboard the ship and provides researchers with the ability to assess the biological efficacy of the BWMS immediately after sample collection. The laboratory provides bench space, fume hood, refrigerator/freezer storage, high grade distilled water and a wet sink area. Ballast-related instruments installed in the ship's lab and utilized for testing included:

- | | |
|---|--|
| • Accuri C6 flow cytometer; | • Microcentrifuge; |
| • Spex Fluoromax-2 high-sensitivity spectrofluorometer; | • YSI portable temperature/salinity meter; |
| • Turner Designs 20/20 ATP luminometer; | • Zooplankton nets (8), 35 µm pore size (50 µm on the diagonal); |
| • Turner Designs TD700 filter fluorometer; | • Zeiss epifluorescence microscopes (2); |
| • Beckman DU530 spectrophotometer; | • Olympus dissecting stereomicroscopes (2); |
| • Beckman Model 73 pH meter; | • IDEXX bacterial MPN tray sealer; |
| • Drying oven; | • Table top centrifuge; |
| • Microbiological culture incubator; | • Filtration racks (2); |
| • Peltier-controlled temperature incubator (warm/cold) for live plankton; | • All pipettes/labware required for sample processing; and |
| | • Walz WaterPam fluorometer. |

GBF personnel are trained on health and safety, information provided in the project TQAP, BWMS operation, and BWMS sample processing, as appropriate. See Appendix C for Project Training Records.

Off-site laboratories were also utilized to process and analyze samples. Particulate organic carbon (POC) and total suspended solids (TSS) samples were filtered in the *Golden Bear* laboratory and then transferred to Moss Landing Marine Labs (MLML) for analysis. MLML is equipped with a Control Equipment Corp. Model 440 Elemental Analyzer (CHN analyzer, or Carbon-Hydrogen-Nitrogen analyzer) for analysis of POC samples. TSS samples were processed at MLML on a calibrated Mettler balance with 0.01 mg resolution.

4 BALLAST WATER MANAGEMENT SYSTEM DESCRIPTION

The Trojan Marinex™ BWT 250 is designed to treat marine water at flow rates up to 250 m³/hr. The Trojan Marinex™ BWT 250 employs a three-step treatment process. The first step of the treatment process utilizes an automatic filter and backwashing system to remove organisms $\geq 50 \mu\text{m}$ during ballast water uptake (ballasting) and pre-treat the water for the second step of the treatment process, UV disinfection. The third treatment step occurs during ballast water discharge (deballasting) when ballast water bypasses the filtration system and is directed through the UV portion of the BWMS for a second dose of UV disinfection before going overboard (see Figure 2).

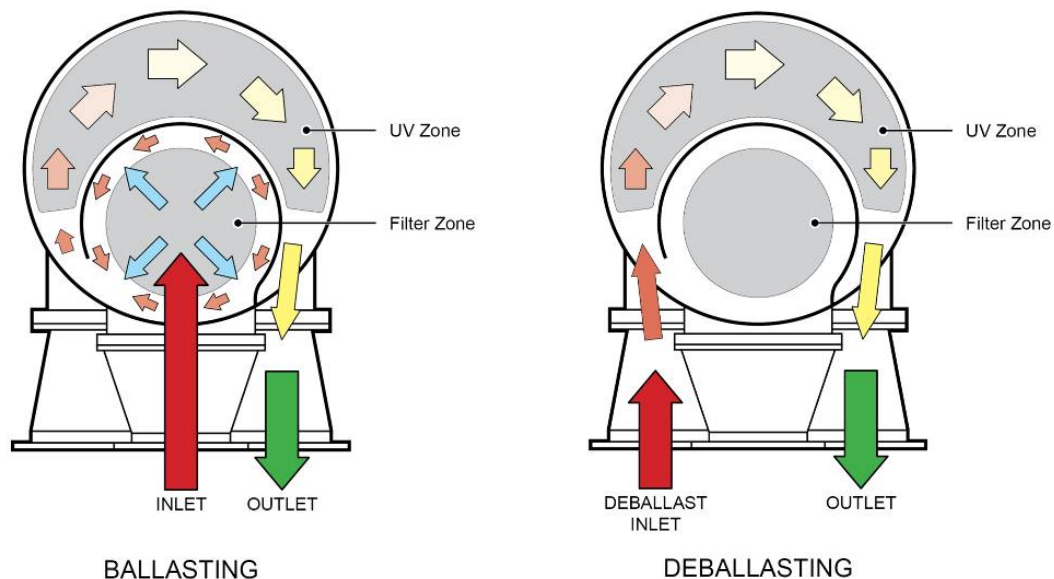


Figure 2 - Ballasting and Deballasting Process Flow

4.1 Ballasting Operation

Ballast water is pumped into the ballast inlet at the top of the treatment unit. The ballast water flows first through the filter elements where debris and larger organisms are removed. An automatic, on-line backwashing cycle removes debris trapped on the filter elements and directs it back to the original source water through the backwash outlet.

Filtered ballast water passes through the filter elements and flows through the UV treatment zone within the unit. The treated water is then directed to the ballast water pipe through the ballast water outlet.

4.1.1 Automatic Filter Backwash System

The BWMS is equipped with an automatic filter backwashing system that automatically initiates a cleaning sequence based on differential pressure across the filtration system. The filtration system has two pressure sensors, one located on the ballast water inlet and the other on the ballast water outlet. These pressure sensors provide a signal to the control panel to initiate a backwash sequence. The backwash sequence consists of opening an actuated backwash valve and signaling the filter drive motor to make one revolution. Each revolution of the drive motor

allows each filter element to reverse its flow allowing accumulated debris trapped by the filter to be discharged overboard. As each individual filter element is being backwashed, the remaining filter elements continue to process water. Once the filter drive motor has completed its revolution, the backwash valve closes completing the cleaning sequence.

4.1.2 Automatic Lamp Cleaning System

The BWMS is equipped with a lamp cleaning system that automatically initiates a cleaning sequence at the beginning and end of a ballasting cycle. The lamp cleaning system removes any fouling that could build up on the lamp sleeves. The lamp cleaning sequence can also be initiated automatically or manually during operation.

The automatic lamp cleaning system is sequentially driven. Each lamp wiping mechanism consists of a wiper plate, wiping seals and one hydraulic actuator. The hydraulic actuator moves the wiper plate with wiping seals from one end of the lamp sleeve to the other. A cleaning sequence consists of actuating the lamp cleaning system from its home position to the end of the lamp sleeves and back to the home position. The cleaning cycle is finished when the last lamp wiping mechanism reaches its home position.

4.1.3 Control System

The BWMS control system monitors and logs key operational parameters allowing automatic engagement of the filter backwashing system, operation of BWMS valves, warning alarms, fault shutdowns and output of the UV lamps. In the event of low UV output, an alarm condition is initiated to warn of the potential for insufficient ballast water treatment. All logged data is available for downloading.

4.2 Deballasting Operation

Upon deballasting, treated ballast water is sent directly to the UV treatment zone and bypasses the filtration portion of the unit. Ballast water enters through the ballast water inlet on the front of the unit, is directed through the UV treatment zone for a second dose of UV treatment, and is directed back to the ballast water pipe for discharge through the ballast water outlet.

The operator may also elect to use filtration on deballasting in which case the controls should be selected to the “ballast” position and the backwash is directed to a holding tank instead of overboard as directed during ballasting.

5 SHIPBOARD TESTING EXECUTION AND LOGISTICS

Shipboard biological efficacy testing consisted of receiving, installing, and commissioning the BWMS, conducting a series of four shipboard tests, and removing the BWMS from the GBF site. The following sections summarize the shipboard testing approach designed to meet the intent of the guidelines specified in IMO G8.

5.1 Shipboard Test Process

Shipboard Test 1 consisted of three specific ballasting events: a combined uptake event to sample both treatment and control ballast water followed by treatment discharge and control discharge. As requested by DNV, the test plan was revised such that shipboard Tests 2, 3, and 4 consisted of four specific ballasting events: treatment uptake, control uptake, treatment discharge, and control discharge. Testing occurred in March 2012 through March 2013 using ambient water conditions according to the schedule, flow rates, ballast volumes, BWMS UV intensity and energy consumption in Table 3 below. The BWMS UV intensity was measured and recorded by the BWMS during treatment uptake. The BWMS operated under one power setting resulting in stable energy consumption throughout treatment events. Also see the Ballasting Operations Log provided in Appendix D and GBF Automation System Outputs provided in Appendix E.

5.1.1 Tank Cleaning and Pipe Flushing

The treatment and control tanks were inspected, cleaned and flushed prior to shipboard Test 1 in accordance with the GBF TQAP to avoid contamination during biological efficacy testing. Due to concurrent BWMS research and development (R&D) trials (see Section 7.1.3), both treatment and control tanks were also cleaned and flushed prior to shipboard Tests 2, 3 and 4.

The GBF piping system was flushed with approximately 20-30 ppm chlorine bleach (sodium hypochlorite) solution and drained prior to each shipboard test. At least 24 hours before treated ballast water discharge, the piping system was flushed again with dilute sodium hypochlorite solution and drained. Flushing between ballast water uptake and treated ballast water discharge was conducted to avoid organism contamination from the untreated uptake water in the pipes.

5.1.2 Recirculation

Before each uptake event, the BWMS inlet piping was primed by recirculating ballast water in sea-to-sea mode. This allowed the BWMS UV lamps to warm up for proper treatment and enabled the sampling team to set-up the sampling system. When the BWMS was ready for treatment, ballast water was redirected from the overboard to the treatment tank.

Recirculation also occurred before treated ballast water discharge to allow the BWMS UV lamps to warm up for proper treatment and enable the sampling team to set-up the sampling system. The piping system was arranged to take suction from the treatment tank and treated ballast water was recirculated back to the pump suction. When the BWMS was ready for treatment, the recirculation loop was opened to overboard.

Table 3 - Shipboard Test Information

Test	Event & Date (dd-mm-yy)	Start Time (PST)	End Time (PST)	Total Sampling Time (hrs:min)	Mean BWMS Effluent Flow Rate (m ³ /hr)	Total Ballast Volume (m ³) Treatment Tank ^a (3-154-1)	Total Ballast Volume (m ³) Control Tank ^a (3-154-2)	Mean UV Intensity ^b (mW/cm ²)	Mean BWMS Energy Consumption (kW)
1	Treatment & Control Uptake ^{c, d} 17-Mar-12	12:59	14:49	1:49	231.8	416.5		8.5	-
	Treatment Discharge 22-Mar-12	12:08	13:00	0:52	240.6 (Stripping 54.0)	182.1	-	-	12.67
	Control Discharge 22-Mar-12	14:59	15:57	0:58	249.4 (Stripping 53.7)	-	194.6	-	-
2	Treatment Uptake 1-May-12	17:41	18:33	0:52	242.7	203.7	-	10.0	12.68
	Control Uptake 1-May-12	18:34	19:26	0:52	251.4	-	212.9	-	-
	Treatment Discharge 6-May-12	13:45	14:39	0:54	244.4 (Stripping 57.8)	187.1	-	-	12.68
	Control Discharge 6-May-12	15:23	16:23	1:00	249.9 (Stripping 51.7)	-	209.2	-	-
3	Treatment Uptake 7-Oct-12	12:54	13:58	1:04	205.3	217.8	-	9.4	12.66
	Control Uptake 7-Oct-12	14:16	15:06	0:50	248.8	-	205.1	-	-
	Treatment Discharge 12-Oct-12	13:05	14:02	0:57	241.9 (Stripping 50.1)	181.2	-	-	12.66
	Control Discharge 12-Oct-12	14:30	15:33	1:03	251.7 (Stripping 49.4)	-	196.5	-	-
4	Treatment Uptake 20-Mar-13	12:33	13:24	0:51	250.0	212.7	-	9.2	12.67
	Control Uptake 20-Mar-13	13:34	14:24	0:50	261.7	-	218.6	-	-
	Treatment Discharge 25-Mar-13	12:58	13:52	0:54	251.5 (Stripping 48.2)	188.9	-	-	12.66
	Control Discharge 25-Mar-13	14:20	15:17	0:57	253.1 (Stripping 49.9)	-	210.8	-	-

a = Total ballast water discharge volumes will be less than total uptake volumes due to the volume lost during ballast system filling and flushing, sampling system set up, and the volume left in the tank below the suction bell mouth.

b = Means reported were onboard measurements by the BWMS.

c = Treatment and control uptake occurred sequentially and untreated water was sampled continuously as a combined event.

d = One of three BWMS phase current transformers was not connected properly during the Test 1 uptake on 17-Mar-12. The mean BWMS energy consumption for this event was not representative of normal operations and is not reported for this event.

5.1.3 Uptake

During ballast water uptake, untreated ballast water samples were taken upstream of the BWMS on the Main Deck. Next, ballast water uptake was treated and directed into the treatment tank. During shipboard Test 1, once the treatment tank reached its designated volume, ballast water uptake was immediately directed to the control tank and the BWMS was shut down. When the control tank reached its designated volume, ballast pumping stopped. During shipboard Tests 2, 3 and 4, the ballast pump and BWMS were stopped at the end of treatment uptake to isolate the treatment tank; valves were realigned to bypass the BWMS; and ballast water uptake to the control tank was initiated as a new event.

A target BWMS effluent flow rate of 250 m³/hr was used for all test events based on the treatment capacity of the Trojan Marinex™ BWT 250. During uptake events, this target flow rate was maintained when the BWMS was not in a backwash cycle. The total ballast water flow during the backwash cycle (backwash flow plus effluent flow) would approach the capacity of the ballast pump typically causing the instantaneous effluent flow rate to drop below target effluent flow rate. The backwash cycle occurred as frequently and for the duration needed to maintain a low pressure differential across the filters (see Figures 3 - 6).

5.1.4 Holding Time

Filled control and treatment tanks were held for the duration of 120 hours +/- 10%. Holding time was measured from the start of the uptake event to the start of the treatment tank discharge event. Control tank discharge was started as soon as possible after the completion of treatment tank discharge.

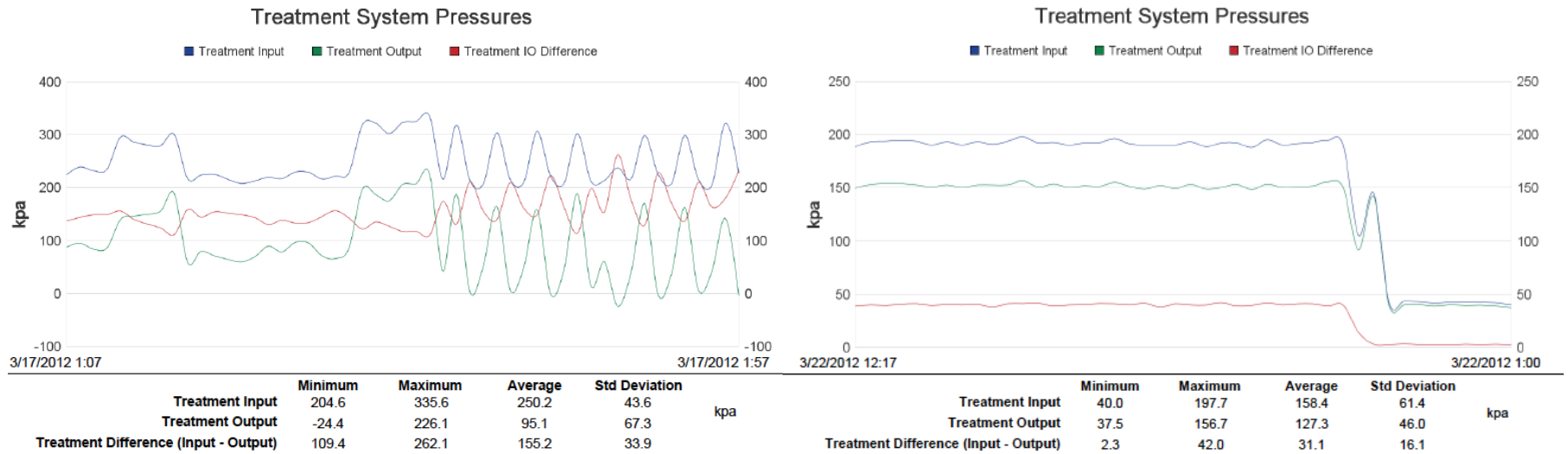


Figure 3 - Test 1 Pressure Measurement Across BWMS Filter; a) Treatment Uptake; b) Treatment Discharge

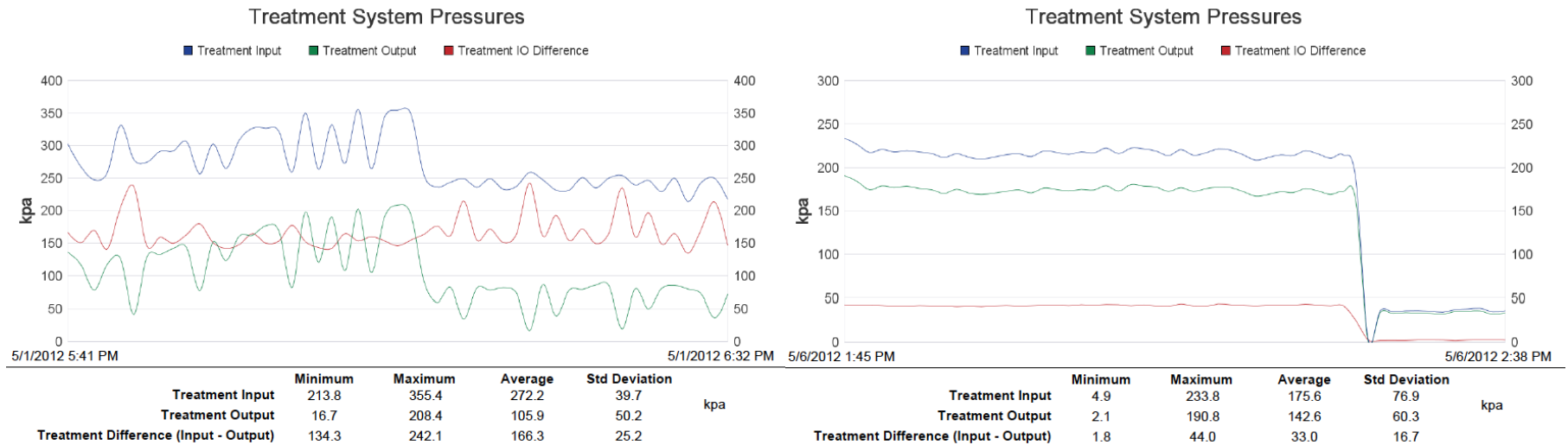


Figure 4 - Test 2 Pressure Measurement Across BWMS Filter; a) Treatment Uptake; b) Treatment Discharge

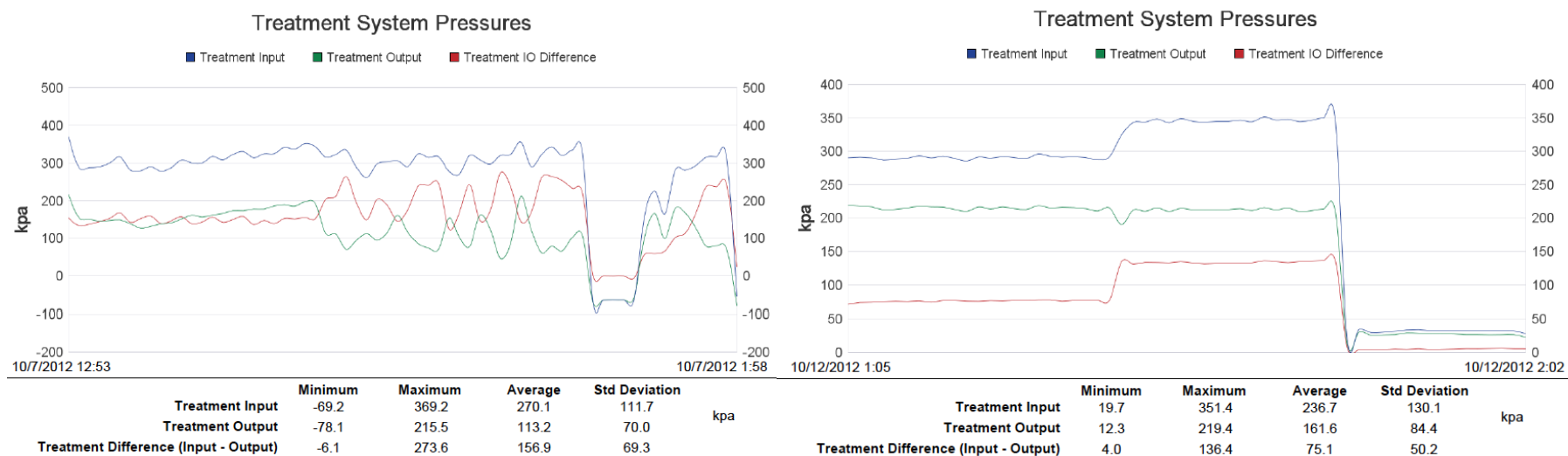


Figure 5 - Test 3 Pressure Measurement Across BWMS Filter; a) Treatment Uptake; b) Treatment Discharge

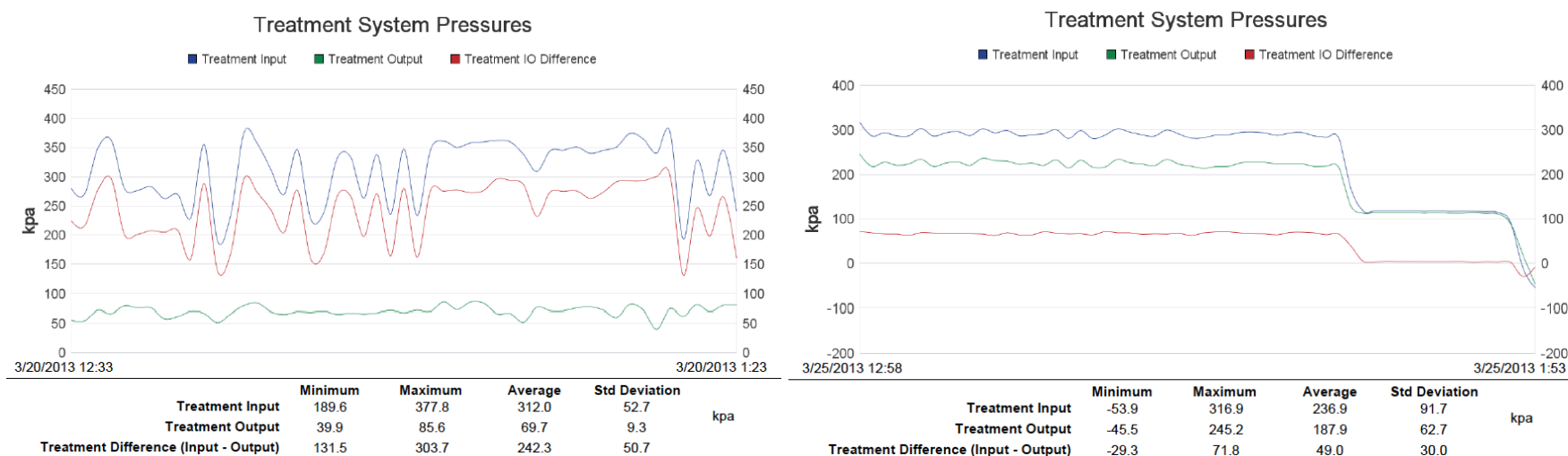


Figure 6 - Test 4 Pressure Measurement Across BWMS Filter; a) Treatment Uptake; b) Treatment Discharge

5.1.5 Treatment Discharge

The GBF piping system was flushed with approximately 20-30 ppm sodium hypochlorite and drained before treated ballast water discharge (see Section 5.1.1 above). The treatment tank was discharged first to avoid piping and sample contamination from the control tank discharge. Sampling took place downstream of the BWMS on the Main Deck.

Ballast water discharge flow rate was maintained at or above 250 m³/hr for over 90% of each discharge event. GBF utilized ship's ballast tanks with internal framing within them. As the ballast water level decreased during discharge, drainage towards the suction point slowed as ballast water passed through the bottom framing. In order to avoid early loss of the ship's ballast pump suction and to maximize the amount of discharge from each tank, the flow rate was reduced near the end of each discharge event for ballast tank stripping. The flow rate during stripping is not included in the average provided in Table 3, but is listed separately to demonstrate that high ballast water flow rates were maintained for most of the discharge event.

GBF monitored the tank level indicator and when one foot of ballast remained in the tank, the ballast pump was slowed. GBF throttled the discharge pinch valve to allow stripping of the tank, maintain sufficient pressure to keep the BWMS and UV lamps flooded, and maintain sample flow to the sampling system. Sampling was slowed proportionally to main ballast water discharge flow rate. Upon the first indication of the ballast water pump losing suction, the pump, sampling system, and BWMS were secured.

5.1.6 Control Discharge

The control tank was discharged last to avoid treated ballast water sample contamination. Sampling took place on the Main Deck utilizing a bypass around the BWMS. Ballast water discharge flow rate was maintained at or above 250 m³/hr for over 90% of each discharge event. As described in the Section 5.1.5 above, the flow rate was reduced near the end of the discharge cycle to avoid early loss of ship's ballast pump suction. The flow rate during this time is not included in the average provided in Table 3.

5.2 Sampling Method

Sampling ports were designed and installed in accordance with specifications within IMO *Guidelines for Ballast Water Sampling (G2)*. The typical pitot sample port design is provided in Figure 7 below. The sampling system allowed sampling of ballast water on uptake before treatment, ballast water discharge after treatment, and control tank discharge. The sampling system included two sets of three polyethylene sub-sample collection tubs that allowed time-integrated samples over the entire uptake and discharge events.

During each treatment and control uptake event, a minimum of three (3) 1.0 m³ ballast water sub-samples were continuously collected in tubs located near the sampling port before treatment for analysis of the ≥50 µm organism size class. At the same time three (3) 20 L carboys were filled, unfiltered, from the same sample

line for analysis of the $<50\ \mu\text{m}$ but $\geq 10\ \mu\text{m}$ organism size class, indicator microbes and water quality.

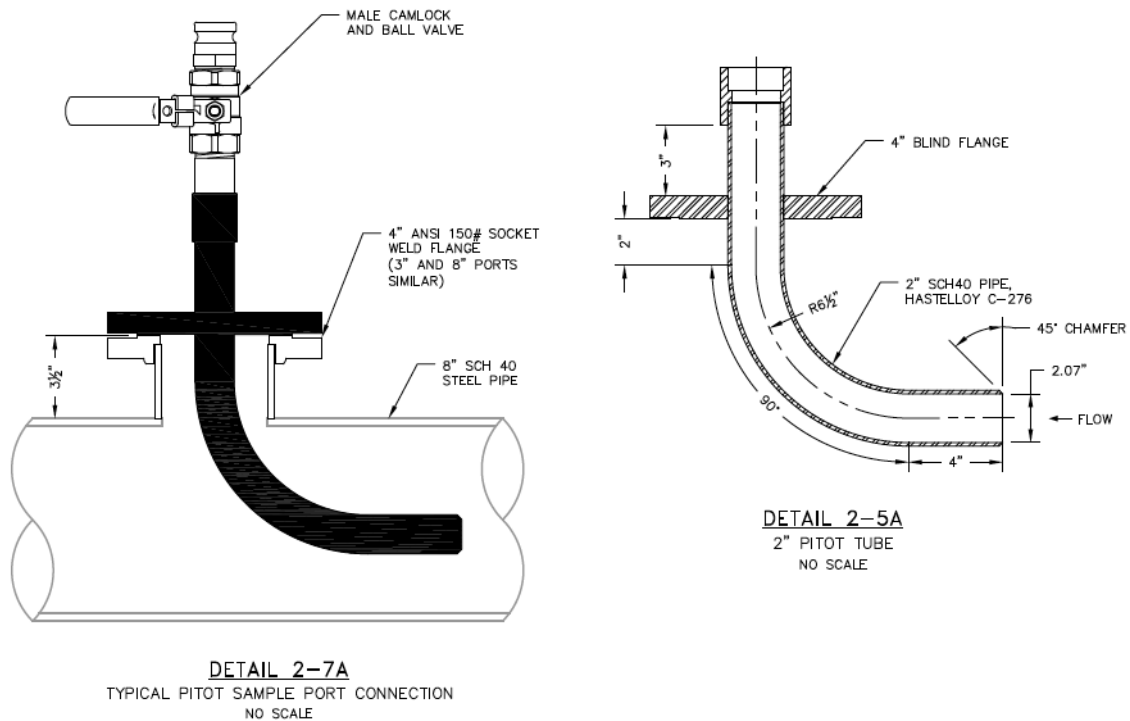


Figure 7 - Typical Pitot Sample Port Design Detail

During each treatment discharge event, a minimum three (3) $3.0\ \text{m}^3$ of ballast water samples were continuously collected for analysis of the $\geq 50\ \mu\text{m}$ organism size class. At the same time three (3) 20 L carboys were filled, unfiltered, from the same sample line for analysis of the $<50\ \mu\text{m}$ but $\geq 10\ \mu\text{m}$ organism size class, indicator microbes and water quality.

During each control discharge event, a minimum of three (3) $1.0\ \text{m}^3$ ballast water sub-samples were collected for analysis of the $\geq 50\ \mu\text{m}$ organism size class. At the same time three (3) 20 L carboys were filled, unfiltered, from the same sample line for analysis of the $<50\ \mu\text{m}$ but $\geq 10\ \mu\text{m}$ organism size class, indicator microbes and water quality.

Organisms $\geq 50\ \mu\text{m}$ were collected by three separate $35\ \mu\text{m}$ mesh zooplankton nets ($50\ \mu\text{m}$ on the diagonal). Each net was suspended in a separate sub-sample collection tub into which a continuous stream of sample ballast water was directed. The sample flow into each sub-sample collection tub was monitored continuously with digital flow meters to provide an accurate determination of the sample volume and maintain isokinetic flow rates at the main sample port pitot tube.

All carboys used to collect sample water for analysis of the $<50\ \mu\text{m}$ but $\geq 10\ \mu\text{m}$ organism size class, indicator microbes and water quality were sealed with lids preventing external contamination of silicone feed tubes which were extended to the bottom of each 20 L carboy to promote gentle fill. Wetted external surfaces (e.g., spigots) were protected with plastic covers (i.e., disposable polyethylene gloves). Figure 8 shows the sampling station and carboys in operation.



Figure 8 - Zooplankton and Microbe Sample Station

There was a small percentage of ballast water that was not sampled. This unsampled volume is estimated at 1-3% of the total ballast water volume based on time required for pipe filling, pipe flushing (to overboard), recirculation, and net initiation time.

5.3 Sample Identification and Labeling

GBF utilized a seven to eight character descriptor for sample identification and labeling. The first four to five characters identified the project test type (CSB for Project Charlie Shipboard) and the chronological number for all BWMS shipboard tests and R&D trials (1-19). The last three characters indicated the event and sample descriptor.

The shipboard tests were interspersed throughout R&D trials and were numbered as they occurred throughout the project. Specifically, shipboard Test 1 was 1st, Test 2 was, 5th, Test 3 was 10th and Test 4 was 19th in project chronology. Shipboard treatment uptake, control uptake, treatment discharge, and control discharge samples were labeled UT, UC, TD and CD, respectively. The three replicate sub-sample tubs for net collections and whole-water microbe collections were labeled A, B, and C. As an example, the complete sample suite for the second shipboard test included the labels CSB-5UTA, CSB-5UTB, CSB-5UTC (Charlie Shipboard Test 5, uptake treatment, 3 sub-samples), CSB-5UCA, CSB-5UCB, CSB-5UCC (Charlie Shipboard Test 5, uptake control, 3 sub-samples), CSB-5TDA, CSB-5TDB, CSB-5TDC (Charlie Shipboard Test 5, treatment discharge, 3 sub-samples), and), CSB-5UTA, CSB-5UTB, CSB-5UTC (Charlie Shipboard Test 5, control discharge, 3 sub-samples). This test numbering sequence was used to identify and label all raw data in Appendix F. However, for reporting and presentation purposes, shipboard test results and tables within Section 8.1 have been shortened to identify the shipboard test number and event.

6 SUMMARY OF ANALYTICAL METHODS

Detailed procedures for each analytical method are included in the Standard Operating Procedures (SOPs) within the TQAP Volume IV (see Appendix A). A summary of all analytical methods used to evaluate ballast water during shipboard testing are outlined below.

6.1 Biological Efficacy

Multiple methods were used to accurately evaluate UV treatment efficacy. The well-documented mode of action for UV treatment is deoxyribonucleic acid (DNA) damage. DNA damage results in failure of cellular replication (reproduction); thus, the latent outcome from UV treatment requires temporal observations of organism concentrations for verification of reproductive failure. Outwardly, the organism may not visually appear damaged, or dead; metabolically the organism may exhibit normal cellular processes such as respiration or enzyme activity. However, when UV treatment is successful, the organism will be rendered non-viable and incapable of reproduction. Additionally, organisms in the $<50\ \mu\text{m}$ but $\geq 10\ \mu\text{m}$ size class are difficult to enumerate quantitatively because live and dead organisms often cannot be distinguished visually. Therefore, multiple required and corroborative methods were used to determine both aspects of UV treatment through live/dead counts and population growth evaluation.

6.1.1 Organisms $\geq 50\ \mu\text{m}$

Organisms $\geq 50\ \mu\text{m}$ in minimum dimension were collected in zooplankton nets according to sampling methods summarized in Section 5.2 above. After treatment and control uptake, contents from each net were transferred to clean, wide-mouth glass jars to a final volume of 200-400 mL (measured volumetrically) depending on the expected organism density and sediment load. Samples were maintained at ambient water temperature. Sample aliquots of 5 mL were placed in a Bogorov serpentine counting chambers and observed under a stereo microscope; nominally, at 30x magnification for determination of live/dead counts. Live organisms were identified by swimming motions, internal movement, or escape responses upon probing. Dead organisms were identified if no activity or movement of any kind was observed, or if organisms were not intact. Live and dead counts of organisms $\geq 50\ \mu\text{m}$ were completed within three hours of sample collection.

6.1.2 Organisms $<50\ \mu\text{m}$ but $\geq 10\ \mu\text{m}$

Organisms in the $<50\ \mu\text{m}$ but $\geq 10\ \mu\text{m}$ size class were sampled as whole water (undiluted/unconcentrated) samples in 20 L carboys. When required, a size fractionation technique was used to isolate organisms in this size class for assays listed below.

Chlorophyll-based Most Probable Number (MPN). Photoautrophic growth was measured with 5 x 8 (replicates x dilutions) MPN matrices incubated under continuous irradiance (approximately $80\ \mu\text{Mole Photon m}^{-2}\ \text{s}^{-1}$) at $15\ ^\circ\text{C}$ for a two week observation period. The length of observation was required to detect responses from the most dilute MPN preparations. Each row of the MPN matrix represented a six-fold quantitative dilution of the preceding row; the first row was

sample water at ambient concentrations of organisms $\geq 10 \mu\text{m}$ followed by dilution-rows. The last row was blank seawater media which served as the dilutant (Guillard's F/2 media). The replicate/dilution range of the matrix provided resolution ranging from minimum levels of $<0.033 \text{ cells/mL}$ to $>5,000 \text{ cells/mL}$. Daily fluorometric analysis of MPN arrays was completed throughout the two week period.

Flow Cytometric Analysis of Live Cells (Corroborative). Flow cytometry was used to count live organisms in whole water samples immediately after collection on the basis of positive cell-specific responses to the live cell marker, fluorescein diacetate (FDA). FDA is a colorless compound which, when metabolized intracellularly by living organisms, yields a measureable green fluorescence signal. The $<50 \mu\text{m}$ but $\geq 10 \mu\text{m}$ size class was defined by inert fluorescent bead standards in relation to the Forward Scatter (FSC) cytometer signal.

C-14 Primary Production (Corroborative). Physiological measurements of photosynthesis (carbon fixation rates) were conducted by incubating triplicate 125 mL whole water samples under continuous illumination for a nominal 24 hour period at 13°C in the presence of C-14 ($2 \mu\text{Ci}$). Whole water sample aliquots were harvested onto GF/F filters ($0.7 \mu\text{m}$) and $10 \mu\text{m}$ nylon filters to estimate total and organisms $>10 \mu\text{m}$ photosynthetic rates, respectively ($\mu\text{gC L}^{-1} \text{ d}^{-1}$). Total dissolved inorganic carbonate (DIC) was determined on a UIC CM5012 CO_2 Coulometer for proper determination of DIC specific activity (dpm/gC). Chlorophyll specific photosynthetic rates were computed from Chlorophyll α measurements made on the same water samples.

Variable Chlorophyll-fluorescence (Corroborative). Phytoplankton variable chlorophyll-fluorescence measurements were made with a Walz pulse amplitude modulated (PAM) fluorometer (WaterPAM) using quartz cuvetts with continuously-stirred 2.5 mL whole water samples. Whole water samples were run through a standard light curve-and-recovery experiment lasting 12 minutes per sample. Dark-adapted samples (>10 minutes dark) were subjected to a rising actinic irradiance (augmented every 30 seconds, after an interrogating saturating flash) from $0\text{-}2567 \mu\text{Mole Photon m}^{-2} \text{ s}^{-1}$. Dark recovery was monitored with geometrically-spaced saturating flashes over approximately six minutes.

Size-fractionated Analysis of Adenosine Triphosphate (ATP) (Corroborative). ATP was measured from 65-280 mL samples harvested onto $7 \mu\text{m}$ and $10 \mu\text{m}$ nylon filters; ATP extraction from the particulate sample was achieved in boiling Tris-buffer (20 mM). Reagents for the calibration and measurement of ATP, by means of luciferin-luciferase luminescence detection, were derived from Promega, Inc. (Enliten ATP kit).

6.1.3 Indicator Microbes $<10 \mu\text{m}$

Escherichia coli by Most Probable Number (MPN). Samples (100 mL) were taken from each replicate 20 L acid-washed carboy for *Escherichia coli* enumeration (Colilert) using IDEXX kits with 24 hour, 35°C dark incubation periods. Three replicate samples from each carboy were analyzed for Test 1 yielding a total of nine (9) sample assays for each ballasting event. This replication rate was determined to be excessive since the test results were below the IMO D-2 standard regardless

if water was treated. Thereafter, one sample from each of three replicate carboys was analyzed for Test 2, 3, and 4. The same reduction in replicate assays was applied to enumeration of *Enterococci* (see below). Analyses provided minimum detection levels of <0.33 CFU/mL and maximum levels >200 CFU/mL.

Enterococci by Most Probable Number (MPN). Samples (100 mL) were analyzed using IDDEX kits for *Enterococci* enumeration (Enterolert) with 24 hour, 35 °C dark incubation periods; the same replication protocol was used as described above for *E. coli*. Analysis provided minimum detection levels of <0.33 CFU/mL and maximum levels >200 CFU/mL.

Vibrio cholerae, Serotypes 01 and 0139. *Vibrio cholerae* was evaluated using two immunospecific detection kits from New Horizons Diagnostics Corp. (Cholera Smart II). Three replicate 100 mL samples from each 20 L acid-washed carboy were harvested onto 0.45 µm pore membrane filters, grown in *V. cholera* alkaline peptone growth media for 48 hours at 35 °C, and challenged colorimetrically against antibodies specific to the antigen lipopolysaccharides of serotype 01 and 0139 (two separate kits). Negative responses indicate ambient concentrations of live *V. cholerae* <1 CFU/100 mL.

Heterotrophic Plate Counts (HPC) (Corroborative). Replicate sterile marine agar plates were spread with whole water samples and dark-incubated at room temperature for 24 hours to evaluate total colony forming units (CFU/mL) from extant cultivable bacteria. Three replicate 50 µL samples from each carboy were analyzed for Test 1. Three replicate 100 µL samples from each carboy were analyzed for Test 2, 3, and 4. Plate counts were derived from digitized images on a BioRad Fluor S-Max Imager.

6.2 Water Quality

The challenge water was characterized by measurement of temperature, salinity, dissolved oxygen, pH, total suspended solids (TSS), particulate organic carbon (POC), dissolved organic carbon (DOC), chlorophyll α (chl α) and UV transmittance according to the methods summarized in Table 4 below. For some measurements, the GBF Automation System and in-situ field measurements were redundant and verified against each other for quality control.

Table 4 - Challenge Water Sample Parameters and Processing Methods

Parameter	Sample method / volume	Processing	Sample Storage	Analysis	Notes
Temperature, salinity, dissolved oxygen	YSI 6600 in-situ probe unit	Immediate	Not applicable	Meter readings, in-situ probe unit	Calibrated at YSI approved facility (Equipco, Concord, CA)
Temperature	GBF Automation System in-line field sensors	Immediate	Not applicable	Meter readings, in-line sensors	None
POC	2 L polypropylene bottle	Volumetric filtration onto pre-combusted GF/F filter	Dried at 65 °C for 48 h, stored in vacuum dessicator@ room °C, analyzed within 30 d	CHN combustion analysis	Combustion analysis on CEC 440 CHN Analyzer (MLML)
DOC	20 mL acid-washed glass vial	Water passed through GF/F filter, directly into glass sample container for storage	Frozen -20 °C, analyzed within 30 d	High temperature catalytic oxidation	Contract analysis (McCampbell Analytical, Inc., Pittsburg, CA; EPA approved laboratory)
TSS	2 L polypropylene bottle	Volumetric filtration onto pre-weighed 0.5 µm membrane filter	Dried at 85°C for 3 hrs, stored in vacuum dessicator @ room °C, analyzed within 30 d	Gravimetric weight determination after drying to constant weight, ± 0.1 mg	Weighed on 5-digit balance, granite weighing table (MLML)
Chlorophyll α	1 L amber polypropylene bottle	Volumetrically harvested onto 25 mm GF/F filter, immediately extracted in 1.2 mL 90% acetone	90% acetone extracts stored @ -20 °C, analyzed within 30 d	Solvent extraction, fluorometric assay for chl α; C-8 HPLC for chlorophylls and carotenoids	Turner TD-700 filter fluorometer, calibrated with HPLC-purified authentic chl α standard (Welschmeyer 1990)
pH	135 mL polypropylene bottle	Within 6 hours of collection	Room °C	Beckman Model 70 pH meter with ThermoOrion Ross combination electrode	Two-point pH standardization
UV Transmittance	20 mL glass vials	Whole water samples analyzed within 12 hours of collection	Room °C	Light receiver at frequencies from 370 nm to 650 nm, and distances of 10 cm and 25 cm	None

7 MODIFICATIONS TO TEST METHODS AND SAMPLING PROCEDURES

The test methods and sampling procedures used to evaluate BWMS treatment efficacy and water quality followed the Project Plan (Plan), QAPPs, and SOPs in the TQAP when possible. In several cases, modifications that did not affect test validity according to IMO G8 criteria were made to the TQAP due to operating or test conditions or at the request of the manufacturer. All modifications to test methods and sampling procedures were either recorded in the revised and red-line version of the TQAP provided in Appendix A or red-lined in the hand-log and hard-copy SOP utilized in each test. The sections below provide a summary of the primary modifications to the overall Project Plan that affected all shipboard SOPs and modifications that affected specific shipboard tests.

7.1 Project Plan Execution

Project modifications below produced a shift in procedure or execution that involved all four or all remaining shipboard tests at the time of execution. These modifications were reported to the type approval administration, DNV, and typically involved a written acceptance of the modification and/or an official issuing of a revised red-line version of the TQAP.

7.1.1 Separation of Treatment and Control Uptake Events

The first version of the shipboard QAPP and SOPs indicated each shipboard test would include a combined uptake event with sequential uptake of ballast water to the treatment tank and then control tank. Shipboard Test 1 was performed with a single, sequential uptake with one sampling event. At the request of DNV after shipboard Test 1 was conducted, a modification to the TQAP was made to separate treatment and control uptake events. The modification was made to capture changes in water conditions due to tidal activity or ship movement during uptake sampling. The revised TQAP Rev. A was followed during shipboard Tests 2, 3 and 4.

7.1.2 Shift in Shipboard Test Locations

All approved TQAP versions included four shipboard tests with uptake in four distinct geographic locations. The shipboard QAPP included uptake in marine, brackish, and fresh water. Modifications to uptake locations or shipboard test postponement were requested by the BWMS manufacturer and resulted in all four test uptake events being performed in the brackish water of central San Francisco Bay, California. The location and schedule changes are outlined below under the individual test.

7.1.3 Cancellation of Land-based Tests

The shipboard tests were originally part of a larger project outlined in the Project Plan of the TQAP that included both shipboard and land-based tests. The land-based tests were cancelled at the request of the BWMS manufacturer. An extended period of the manufacturer's R&D trials took place instead of the land-

based tests. Biological efficacy and water quality results gathered during R&D trials will not be provided within this report.

7.2 Shipboard Test 1

Shipboard Test 1 took place from 17 March 2012 to 22 March 2012 following the QAPP (Shipboard) and SOPs included in the original TQAP. Several modifications to procedures occurred or were initiated during Test 1 as recorded in the revised TQAP Rev. A (9 April 2012) and summarized below.

7.2.1 Separation of Treatment and Control Uptake Events

Treatment and control uptake was treated as one continuous event with a single set of samples. DNV and GBF discussed concerns regarding effects of tidal changes or vessel movement on samples over the uptake duration. These discussions initiated a revision to the TQAP issued as Rev. A (9 April, 2012) after Test 1 was conducted. Since DNV approved the original TQAP prior to start of Test 1, it was agreed this shipboard test would be accepted and valid so long as control uptake and discharge met challenge water criteria. Organism concentrations in Test 1 control discharge were within the range of organism concentrations in control discharge of subsequent tests. It is assumed that modified uptake procedures had no effect on biological efficacy results.

7.2.2 Sample Identification and Labeling

Sample identification and labeling was slightly modified for shipboard Test 1 using a single letter to designate the event uptake, treatment discharge, and control discharge (i.e., U, T, C). Subsequent tests used a two character designator (i.e. UT, UC, TD, CD) to label samples for treatment uptake, control uptake, treatment discharge, and control discharge.

7.2.3 Clock Adjustment

The GBF Automation System clock was behind by eight minutes in shipboard Test 1. The clock error was discovered after start of the Test 1 uptake, was maintained throughout Test 1 for consistency, and adjusted to the correct time for all subsequent tests. Test times recorded in the Automation System Outputs have been corrected for presentation within this report (see Appendix E).

7.2.4 GBF Valve Correction

SOP 15, Operation of BWTS and Facility Piping System (SHIPBOARD), Steps 1, 2, 5, and 6 were revised to throttle valve “L” instead of valve “K”. This typographical error was red-lined in the TQAP Rev. A (9 April, 2012) by the ballast system operator.

7.3 Shipboard Test 2

Shipboard Test 2 took place from 1 May 2012 to 6 May 2012 following guidance in the TQAP Rev. A (9 April, 2012) with the following modifications or red-lines to revised procedures.

7.3.1 Tank Cleaning and Pipe Flushing

The BWMS manufacture was concerned with possible contamination of test tanks from concurrent BWMS R&D trials. The Shipboard QAPP and SOPs were modified to include tank cleaning and pipe flushing prior to each test.

7.3.2 GBF Pump and Valve Modification

SOP 15, Operation of BWTS and Facility Piping System (SHIPBOARD), Steps 3 and 4 were red-lined to “stop” the BWMS and ballast pump at the end of treatment uptake, realign valves per SOPs, and start pump and control uptake. The modification to the procedure provides for a safer operation and has no effect on the engineering data or results.

7.3.3 GBF Valve Correction

SOP 15, Operation of BWTS and Facility Piping System (SHIPBOARD), Step 8 was red-lined to close valve “N” instead of “P”. This typographical error was red-lined in the TQAP Rev. A (28 August, 2012) by the trained ballast system operator.

7.4 Shipboard Test 3

Shipboard Test 3 was originally scheduled for 24 June 2012 to 29 June 2012 with uptake in Long Beach, California and discharge in Vallejo, California. Test 3 was cancelled at request of the BWMS manufacturer and rescheduled to be used as the final six month test from 7 October 2012 to 12 October 2012 utilizing procedures per the TQAP Rev. A (28 August, 2012). The following red-lines and modifications to procedures were incorporated for Test 3.

7.4.1 Tank Cleaning and Pipe Flushing

According to the TQAP Rev. A (28 August, 2012), tank cleaning and pipe flushing was conducted prior to shipboard Test 3.

7.4.2 Filtration on Treatment Discharge

At the BWMS manufacturer’s request, shipboard Test 3 utilized filtration in addition to UV treatment upon treatment discharge with filter backwash effluent diverted to a ballast tank. This modification was conducted to evaluate if treatment efficacy could be improved.

7.4.3 Filtration on Treatment Discharge

The YSI 6600 in-situ probe unit was not activated during the shipboard Test 3 treatment or control discharge events. This resulted in the absence of in-situ temperature, salinity, and dissolved oxygen data as noted in Table 14. However, in-line temperature data was available from the GBF Automation System and is presented in Table 14 and Appendix E.

7.5 Shipboard Test 4

The fourth and final shipboard test was performed 20 March 2013 to 25 March 2013. After cancellation of the land-based testing by the BWMS manufacturer, there was a request by the BWMS manufacturer for a final red-line version of the TQAP and one additional shipboard test that would NOT include filtration on discharge of treated ballast water. A TQAP, Rev. A (16 March, 2013) red-line version was approved by DNV and Test 4 was conducted following modifications or red-lines to test procedures.

7.5.1 Tank Cleaning and Pipe Flushing

According to the TQAP Rev. A (28 August, 2012), tank cleaning and pipe flushing was conducted prior to shipboard Test 3.

7.5.2 Filtration on Treatment Discharge

Similar to shipboard Test 1 and 2, shipboard Test 4 did not utilize filtration on treatment discharge. Ballast water treatment included filtration only on uptake.

8 RESULTS

GBF reports here on the scientific results of the biological efficacy evaluation for the Trojan Marinex™ BWT 250 as determined over four consecutive tests in San Francisco Bay, California. Detailed tables and figures showing a summary of all biological efficacy and water quality test results are presented in the following sections. For all raw data for biological efficacy and water quality tests, see Appendix F. Test results were assessed to interpret the degree of acceptability according to methods indicated in the TQAP and all target criteria were met; the resulting data quality indicators are provided in Appendix G.

8.1 Biological Efficacy

The presentation of biological efficacy results below follows the order of analytical methods in Section 6.1 above. Evaluation of live organisms $\geq 50 \mu\text{m}$ was made with the 'poke and probe' visual determination. However, evaluation of live organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ was made with multiple methods. The TQAP indicated that the MPN method served as the primary evaluation method for organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$, with corroboration by supporting methods including 1) FDA-marked live-cell counts by flow cytometry, 2) C-14 estimates of photoautotrophic primary production (carbon fixation rate), 3) variable chlorophyll fluorescence by pulse amplitude modulated (PAM) fluorometry, and 4) size-fractionated ATP analysis. The reason for execution of multiple methods for organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ involves the nature of UV treatment as a sterilization, or 'inactivation', technique (see Section 6.1)

8.1.1 Organisms $\geq 50 \mu\text{m}$

Summary results for numeric counts of live organisms $\geq 50 \mu\text{m}$ are provided in Table 5 and Figure 10. Uptake control and uptake treatment concentrations taken on day-zero of each test ranged from approximately 37,000-118,000 live organisms/ m^3 . Discharge data from control and treatment ballast tanks were generated after the required five day hold time. Control discharge concentrations after five days of dark ballast tank hold-times were roughly half their respective uptake concentrations in 3 of the 4 tests. The decrease in concentration of organisms $\geq 50 \mu\text{m}$ after containment in dark ballast tanks was expected (Gollasch *et al.*, 2000).

Live organism concentrations in all treatment discharge samples were reduced by orders of magnitude more than both uptake and control discharge samples. Shipboard Tests 1, 3, and 4 showed mean live organism concentrations reduced by factors of 4.1×10^4 , 1.2×10^6 , and 2.3×10^4 , respectively, compared to their uptake concentrations. No live organisms were detected in Test 2 implying complete removal of live organisms for the sample volumes evaluated in this shipboard test. All four shipboard tests yielded mean live organism concentrations in treatment discharge that were < 10 live organisms/ m^3 demonstrating compliance with IMO D-2 standard.

The total volumes of treatment discharge samples were 10.03 m^3 , 9.96 m^3 , 9.98 m^3 and 9.76 m^3 for Tests 1, 2, 3, and 4, respectively. Treatment discharge volumes for each test were more than three times the minimum sample volume required by IMO

G8 (3.0 m³). Collectively, 37 live organisms were detected in a total of 40 m³ collected in all tests combined.

The live organisms observed in treatment discharge samples were mostly small tintinnids and copepod nauplii (see Figure 9). See Appendix H for organism composition and taxonomy present in treatment discharge samples.

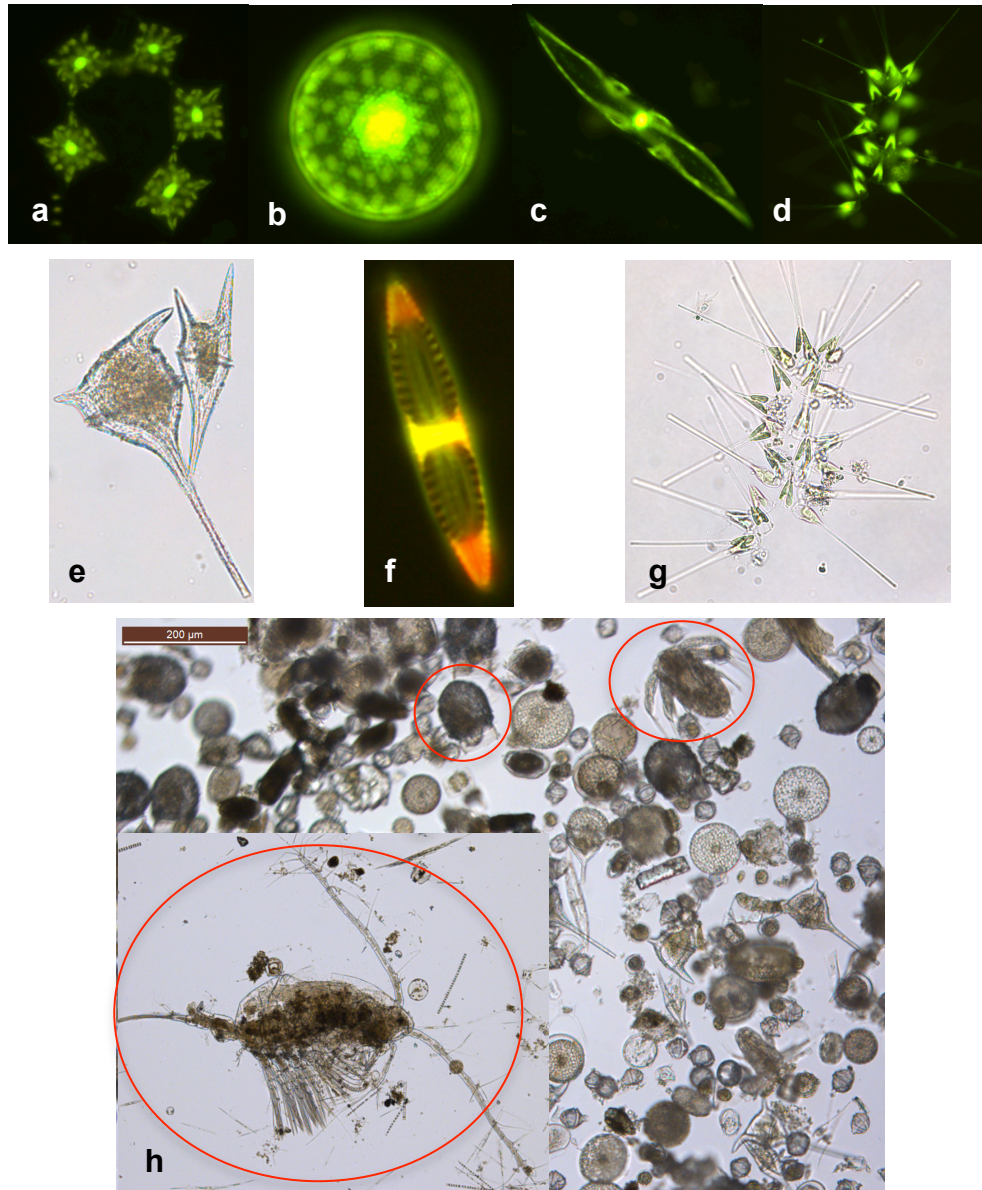


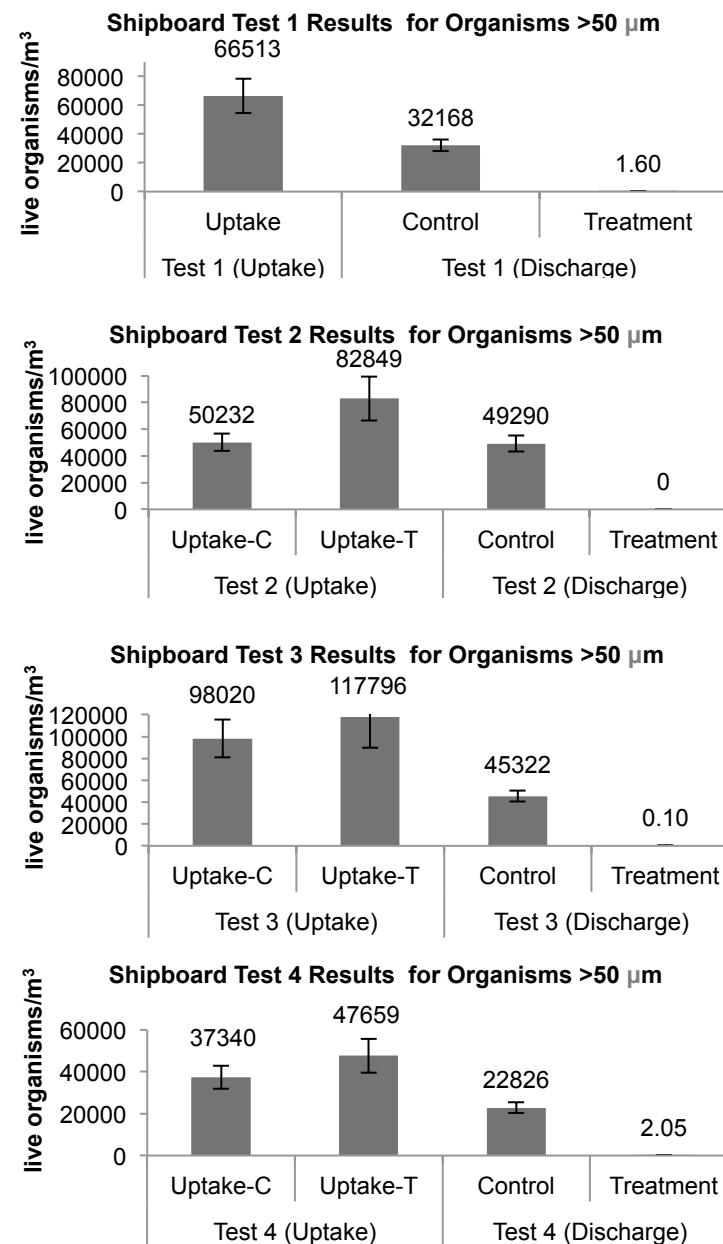
Figure 9 - Common $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ Phytoplankton and $\geq 50 \mu\text{m}$ Zooplankton Observed: a) *Odontella*, b) *Thalassiosira*, c) *Pleurosigma*, d and g) *Asterionella*, e) (left to right) *Ceratium divaricatum* and *Ceratium lineatum*, f) *Navicula*, and h) (circled in red, left to right) adult copepod, tintinnid, and copepod nauplii

Table 5 - Organisms $\geq 50 \mu\text{m}$ Results

Test Event	Sample Type	Sample name	Mean (live organisms/m ³)	S.D. (live organisms/m ³)	C.V. (%)	n
1 (Uptake)	Uptake	1U	66513	11967	18	18
1 (Discharge)	Control	1C	32168	4036	13	18
	Treatment	1T	1.60	0.35	22	18
2 (Uptake)	Control	5UC	50232	6660	13	6
	Treatment	5UT	82849	16327	20	6
2 (Discharge)	Control	5CD	49290	6009	12	18
	Treatment	5TD	0.00	0.00	0	18
3 (Uptake)	Control	10UC	98020	17249	18	18
	Treatment	10UT	117796	28267	24	18
3 (Discharge)	Control	10CD	45322	5013	11	18
	Treatment	10TD	0.10	0.17	173	18
4 (Uptake)	Control	19UC	37340	5458	15	18
	Treatment	19UT	47659	8129	17	18
4 (Discharge)	Control	19CD	22826	2492	11	18
	Treatment	19TD	2.05	0.35	17	14

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples

Figure 10 - Organisms $\geq 50 \mu\text{m}$ Results Comparison (right)



8.1.2 Organisms $\geq 10\ \mu\text{m}$ but $< 50\ \mu\text{m}$

Chlorophyll-based Most Probable Number (MPN). Numeric estimates of organisms $\geq 10\ \mu\text{m}$ but $< 50\ \mu\text{m}$ were determined from MPN culture-based growth methods conducted under continuous irradiance at $15\ ^\circ\text{C}$. The target organisms were photoautotrophs detected by measurements of whole-cell chlorophyll α fluorescence (excitation/emission; 430nm/680nm). The samples were sieved through $10\ \mu\text{m}$ mesh to remove small organisms and resuspended in an equivalent volume of fresh growth media resulting in no net change in the original concentration. Examples of the photoautotrophs that grew in these MPN experiments are given in Figure 11. The cells were generally large but also included some smaller colonial organisms or long, single-celled organisms (pennate diatoms) that were retained by the $10\ \mu\text{m}$ mesh.

The MPN results are summarized in Table 6 and Figure 12. All treatment discharge samples yielded numeric results < 10 cells/mL, thus meeting IMO D-2 standard. Numeric MPN concentrations showed that < 0.06 live organisms/mL were detected in all four shipboard tests; minimum levels specified by ' $<$ ' notation in Table 6 (e.g., < 0.056 cells/mL; Test 3) refer to the fact that no growth was detected in any of the 35 growth tubes interrogated in each of the three MPN matrices analyzed.

In total, more than 1,600 MPN tubes were manually measured for fluorescence confirmation of cellular growth throughout the four shipboard tests. Figure 13 presents a visual summary of the MPN raw results for the logical comparison of control uptake water with treatment discharge water. Note that in Figure 13, Shipboard Test 1 and Test 2 uptake samples were replicated in triplicate (one each from Tubs A, B and C); in Tests 3 and 4 the replicate number was reduced to conserve incubator space. Green squares indicate positive scores for growth in any individual MPN tube; red indicates no growth detected (all MPN matrices were evaluated weekly for a minimum of two weeks to ensure stabilized scores for growth/no-growth; some matrices were monitored for over six weeks). The 5x8 matrix design (40 tubes; 3 mL each) included five replicates for each of seven serially-diluted rows of tubes; the eighth row consisted of uninoculated raw growth media, serving as a blank to determine if growth by contamination would occur.

Dilutions initially included five-fold reductions per row in Test 1, later modified to six-fold reduction per row in Tests 2, 3 and 4, yielding final dilutions of $15625\times$ – $46656\times$, respectively. Figure 13 shows that the designed dilution series met the fundamental objective of general MPN protocol to create an adequately diluted final row that contained no live cells and thus showed no evidence of positive growth (Cochran, 1950; Woomer et al., 1990). Another fundamental assumption of MPN technique is that the target organisms will grow in the media and under growth conditions provided. This was confirmed to be true in all MPN matrices assembled in all four shipboard tests. Specifically, successful growth was confirmed in $> 97\%$ of all tubes where growth was expected. That is, 97% of all tubes in the first 2-4 rows of all *untreated* samples, both uptake and control-discharge, showed positive growth. As seen in Figure 13, this is a conservative estimate of growth success since positive growth was often observed in the fifth dilution row. Note that growth would *not* be expected in the *last* few rows of an untreated sample due to successful live-cell dilution as evidenced in Figure 13. The cells that grew included

autotrophs (single-cell or colonial) that would have been successfully captured by the 10 µm sieve that was used to prepare the raw sample water (see Figure 11).

MPN tubes from treated samples showed little indication of growth at any dilution, as shown in Figure 13. Overall, >97% of all *treatment* discharge tubes scored as no-growth as expected (under the assumption of 'successful' treatment). The final MPN concentration of approximately 0.06 live cells/mL for all four shipboard tests was at least two orders of magnitude lower than the maximum concentration of 10 viable cells/mL as allowed by the IMO D-2 standard (see Table 6).

Flow Cytometric Analysis of Live Cells (Corroborative). Flow cytometric FDA counts of viable organisms ≥ 10 µm but < 50 µm are summarized in Table 7 and Figure 14. The FDA marking protocol yields green fluorescent cells resulting from enzymatic conversion of non-fluorescent FDA to its fluorescent product, fluorescein. Marked reductions of uptake live concentrations were noted in all treatment samples by at least one order of magnitude. However, only Test 3 yielded FDA-based live counts less than 10 live cells/mL meeting IMO D-2 standard.

C-14 Primary Production (Corroborative). Table 8 and Figure 15 present results of C-14 based measurements of photosynthetic primary production ($\mu\text{g C}/(\text{L}\cdot\text{d})$). The organisms ≥ 10 µm but < 50 µm are often presumed to be dominated by phytoplankton (ETV Protocol, 2010). Thus, it is reasonable to make direct measurements of photosynthesis to corroborate whether a given BWMS treatment technology successfully impairs the metabolic process of carbon fixation, the fundamental mode of growth and reproduction for photoautotrophs. The photosynthetic rate is not an absolute indicator of numeric counts but focuses instead on direct metabolic activity. As such, the assay is not meant to evaluate numeric live counts, but it does provide direct interrogation of photosynthetic metabolism, a corroborative factor, integrally related to viability. The data reported below were processed on fine pore GF/F filters (0.7 µm nominal pore size) so that total phytoplankton photosynthesis could be evaluated. This permits comparison of rates derived here to the numerous reports of natural photosynthetic rates in various marine/freshwater environments (Field et al., 1998).

As shown in Table 8 and Figure 15, UV treatment resulted in significant depression of photosynthetic rates in treatment discharge relative to control uptake. Shipboard Tests 1, 2 and 3 showed 100-fold to 400-fold reductions in photosynthetic performance; Test 4 yielded a reduction of approximately six-fold.

Typical 'blue water' C-14 based photosynthetic rates in surface offshore oligotrophic environments are approximately 5 $\mu\text{g C}/\text{L}\cdot\text{day}$ (Fujieki, 2012). Those data are based on 24 hour incubations under natural irradiance, which include a nominal 12 hour dark period. Results in Table 8 were generated from 24 hour experiments with continuous, 24 hour saturating irradiance ($180 \mu\text{Mole Photon m}^{-2} \text{d}^{-1}$) and thus represent generous estimates of daily photosynthetic rates. It appears that UV treatment reduced natural San Francisco Bay photosynthetic rates to values at, or below, the severely oligotrophic offshore marine environment, thus reducing ambient San Francisco Bay phytoplankton concentrations to a condition at least equivalent to ballast water exchange.

Variable Chlorophyll-fluorescence (Corroborative). Results for PAM measurements yielded the primary physiological measurement, F_v/F_m , which represents the

quantum efficiency of photosystem II photochemistry (defined as the fraction of absorbed photons that successfully participate in photosynthetic electron transfer flow). F_v/F_m is expected to yield a maximum dark-adapted value of 0.6-0.7 (dimensionless) for non-stressed, healthy phytoplankton (Maxwell and Johnson, 2000); dead phytoplankton (or algal cells with fully damaged photosynthetic components) are expected to yield F_v/F_m values approaching zero. A pre-programmed rapid light-curve experiment (Maxwell and Johnson, 2000) was used to generate values of F_v/F_m for each sample (determined from the first dark-adapted measurement of F_v/F_m obtained in the dark phase of the rapid light-curve). A 10-12 minute recording of the light-curve response was collected for every sample to provide visual traces of the variable fluorescence response (including multiple determinations of F_v/F_m throughout the rising actinic light incubation and subsequent dark recovery period).

Results for F_v/F_m are given in Table 9 and Figure 16. Untreated uptake samples collected on day-zero of the ballast cycle generally yielded F_v/F_m values in the range 0.6-0.7, as expected. A slight decrease in F_v/F_m was observed for untreated control discharge samples resulting from the five day containment period in the ship's darkened ballast tank. However, all treatment discharge samples from the four shipboard tests showed depressed values of $F_v/F_m < 0.2$. The signals from treatment discharge samples were severely depressed relative to untreated samples and this can best be seen by examining time-based fluorescence traces of the rapid light curve experiments (see Figure 17). UV treatment resulted in a depressed saturating fluorescence pulse (F_m) that was essentially lost within the noise of the instrument background signal (F_o). Compare the flattened *treatment* fluorescence trace with that from the *untreated* traces which all showed clearly-defined saturating fluorescence pulses throughout the first five minutes of the increasing light curve and throughout the ensuing dark recovery period. The noisy, reduced signal of the treatment samples yielded abnormally high coefficients of variation (up to 173%) indicative of F_v/F_m ratios close to zero (see Table 9); the instrument does not report negative values for F_v/F_m .

Treated samples held in the dark and in the light for up to two weeks (data not shown) showed no indication of recovery of the dark-adapted F_v/F_m signal indicating UV treatment resulted in permanent damage to the photosynthetic apparatus.

Size-fractionated Analysis of Adenosine Triphosphate (ATP) (Corroborative). During shipboard Tests 3 and 4, ATP analysis was executed on particles captured on 10 μm pore size filters (see Table 10 and Figure 18). Treatment discharge samples showed ATP levels that were two to three orders of magnitude lower than that of control uptake water. Although ATP is not a direct measurement of numeric cell counts, the values can be converted to equivalent live cell counts based on numerous determinations of ATP content in cells of known cell volume (Karl, 1993). A reasonable value of ATP content for cells of 15 μm equivalent spherical diameter (ESD) is approximately 1 pg ATP/cell. Assuming 15 μm ESD is an appropriate average cell size for organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$, the measured treatment discharge ATP concentrations of 0.07 and 1.52 ng ATP/L for Tests 3 and 4 (see Table 10) represents the equivalent of 0.07 and 1.52 live cells/mL, respectively. Both estimates are less than the maximum IMO D-2 standard of 10 viable cells/mL.

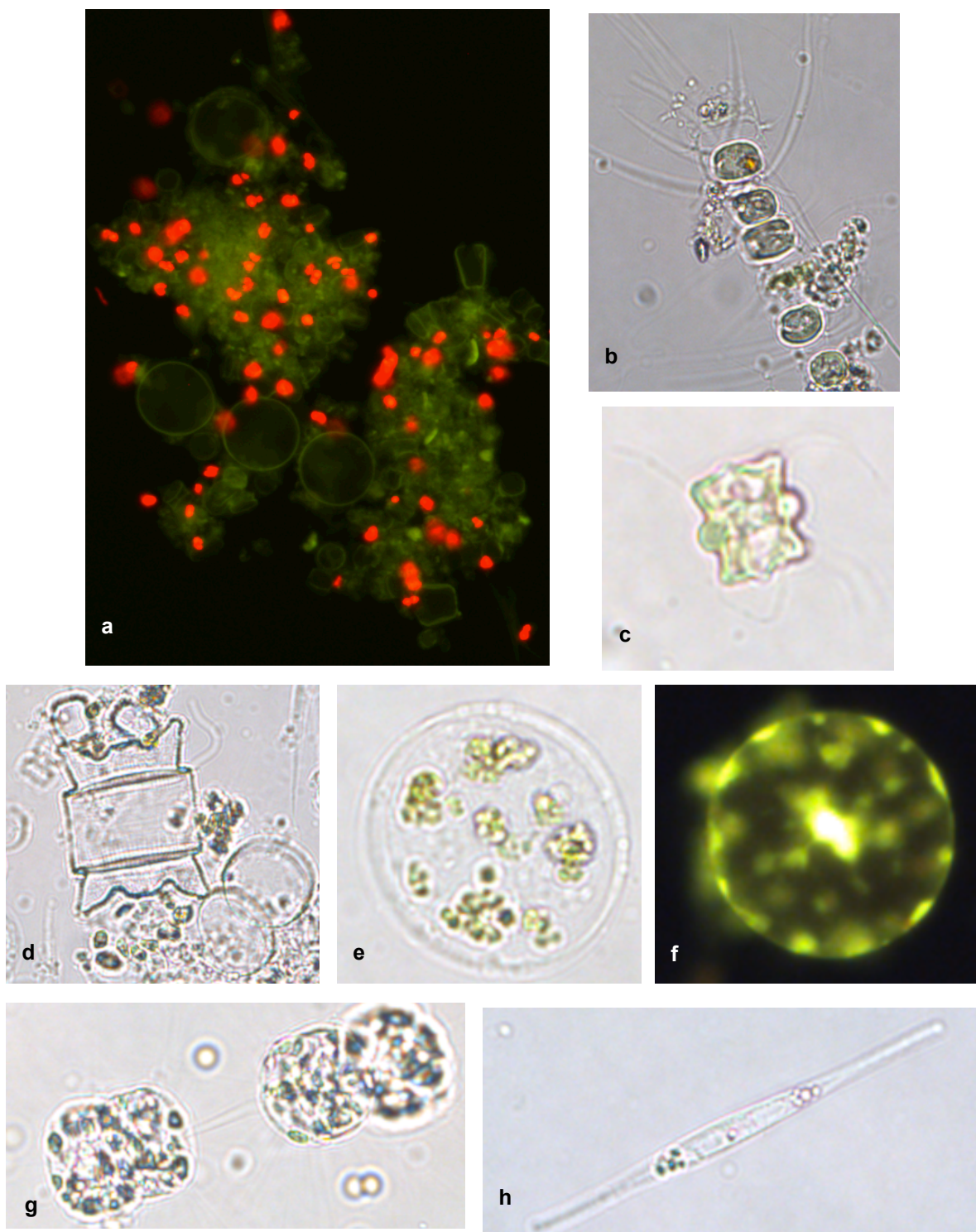


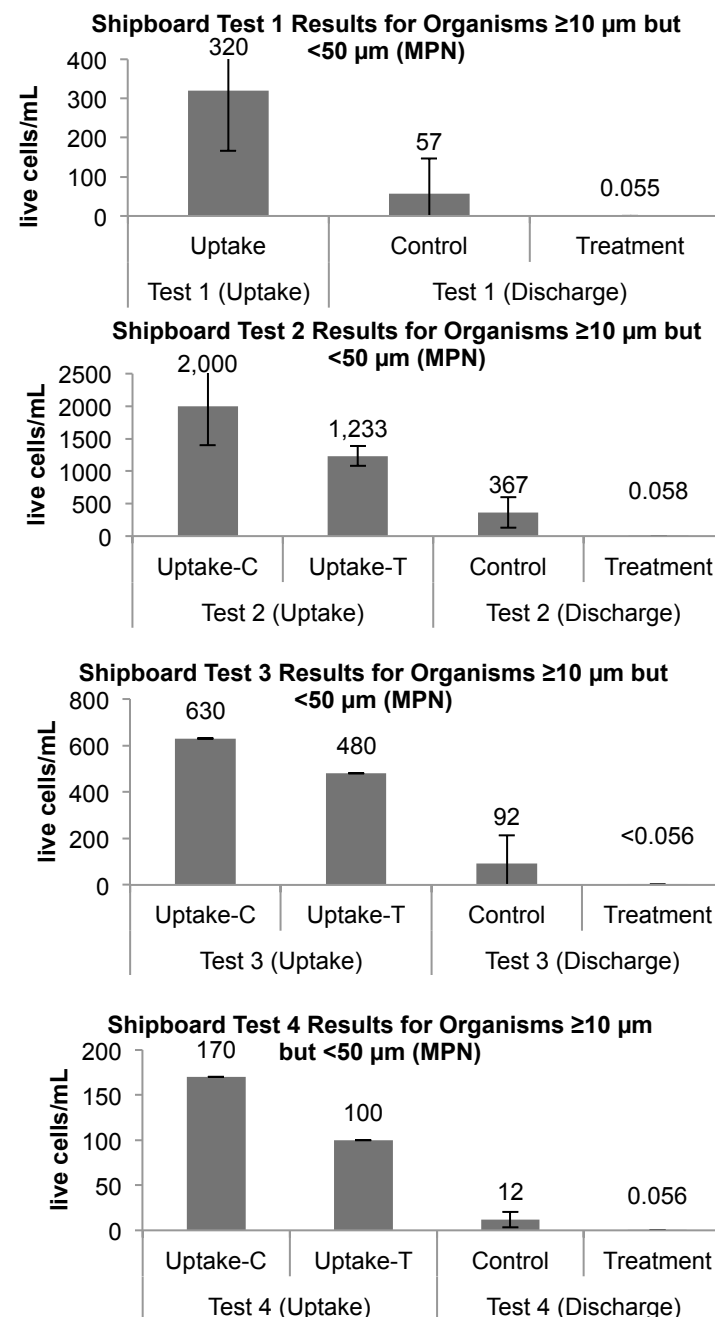
Figure 11 - Phytoplankton Observed in MPN Grow-out Experiments: a) conglomerate of 5 µm autotrophs (red) and diatom tests, b and c) *Chaetoceros*, d) *Odontella*, e and f) *Thalassiosira*, g) *Stephanopyxis* (20µm), h) pennate diatom

Table 6 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, MPN Results

Test Event	Sample Type	Sample Name	Mean (live cells/mL)	S.D. (live cells/mL)	C.V. (%)	n
1 (Uptake)	Uptake	1U	320	154	48	3
1 (Discharge)	Treatment	1T	0.055	0.003	5.28	3
	Control	1C	57	89	157	3
2 (Uptake)	Treatment	5UT	1233	153	12	3
	Control	5UC	2000	600	30	3
2 (Discharge)	Treatment	5TD	0.058	0.003	5.01	3
	Control	5CD	367	237	65	3
3 (Uptake)	Treatment	10UT	480	n/a	n/a	1
	Control	10UC	630	n/a	n/a	1
3 (Discharge)	Treatment	10TD	<0.056	n/a	n/a	3
	Control	10CD	92	121	133	3
4 (Uptake)	Treatment	19UT	100	n/a	n/a	1
	Control	19UC	170	n/a	n/a	1
4 (Discharge)	Treatment	19TD	0.056	0	0	3
	Control	19CD	12	9	73	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples; n/a = not analyzed

Figure 12 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, MPN Results Comparison (right)



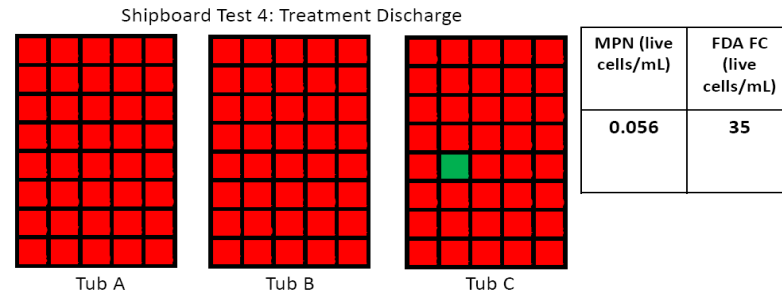
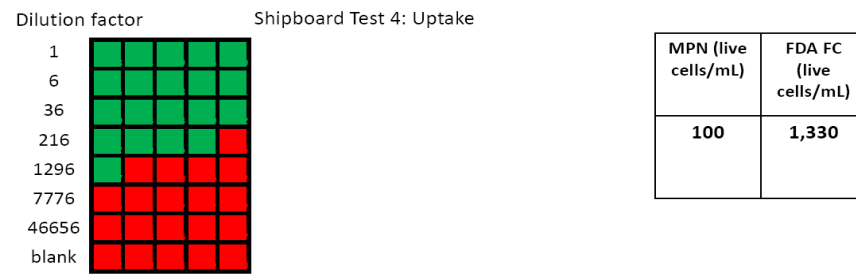
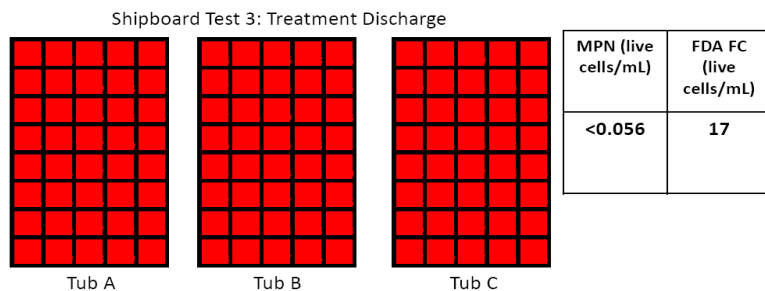
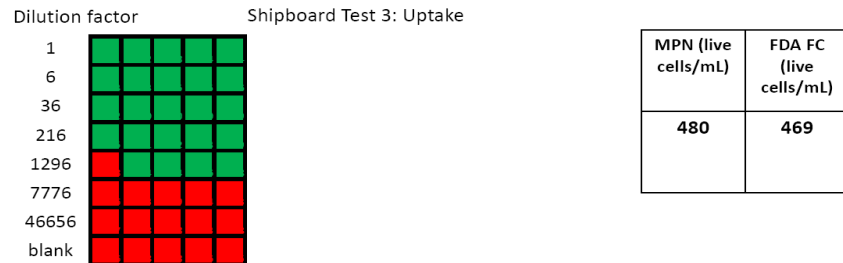
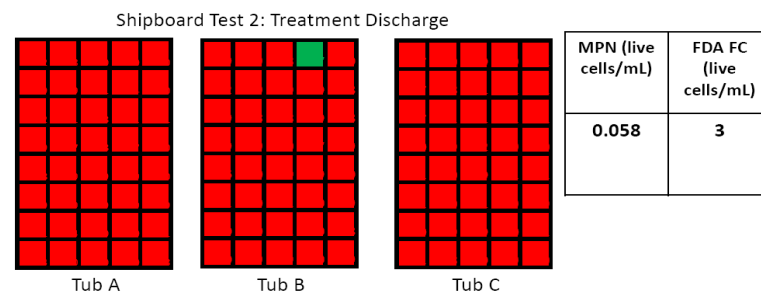
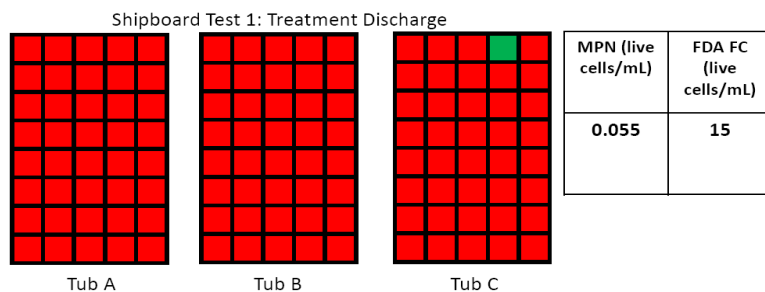
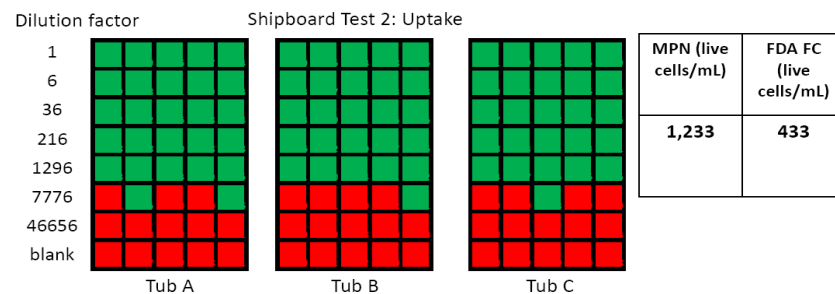
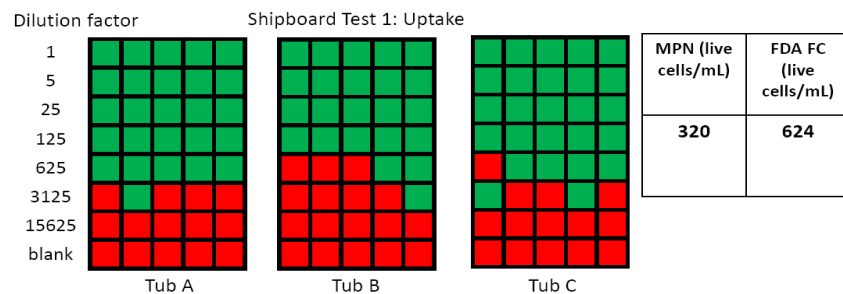


Figure 13 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, MPN Uptake and Treatment Discharge Results

Table 7 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, Flow Cytometry Results

Test Event	Sample Type	Sample Name	Mean (live cells/mL)	S.D. (live cells/mL)	C.V. (%)	n
1 (Uptake)	Uptake	1U	624	108	17	9
1 (Discharge)	Treatment	1T	15	16	103	9
	Control	1C	114	49	43	9
2 (Uptake)	Treatment	5UT	433	59	14	9
	Control	5UC	188	31	17	9
2 (Discharge)	Treatment	5TD	3	5	150	9
	Control	5CD	131	30	23	9
3 (Uptake)	Treatment	10UT	469	85	18	9
	Control	10UC	450	81	18	9
3 (Discharge)	Treatment	10TD	17	17	97	9
	Control	10CD	92	26	28	9
4 (Uptake)	Treatment	19UT	1330	113	11	9
	Control	19UC	1000	180	11	9
4 (Discharge)	Treatment	19TD	35	18	33	9
	Control	19CD	306	53	17	9

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples

Figure 14 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, Flow Cytometry Results Comparison (right)

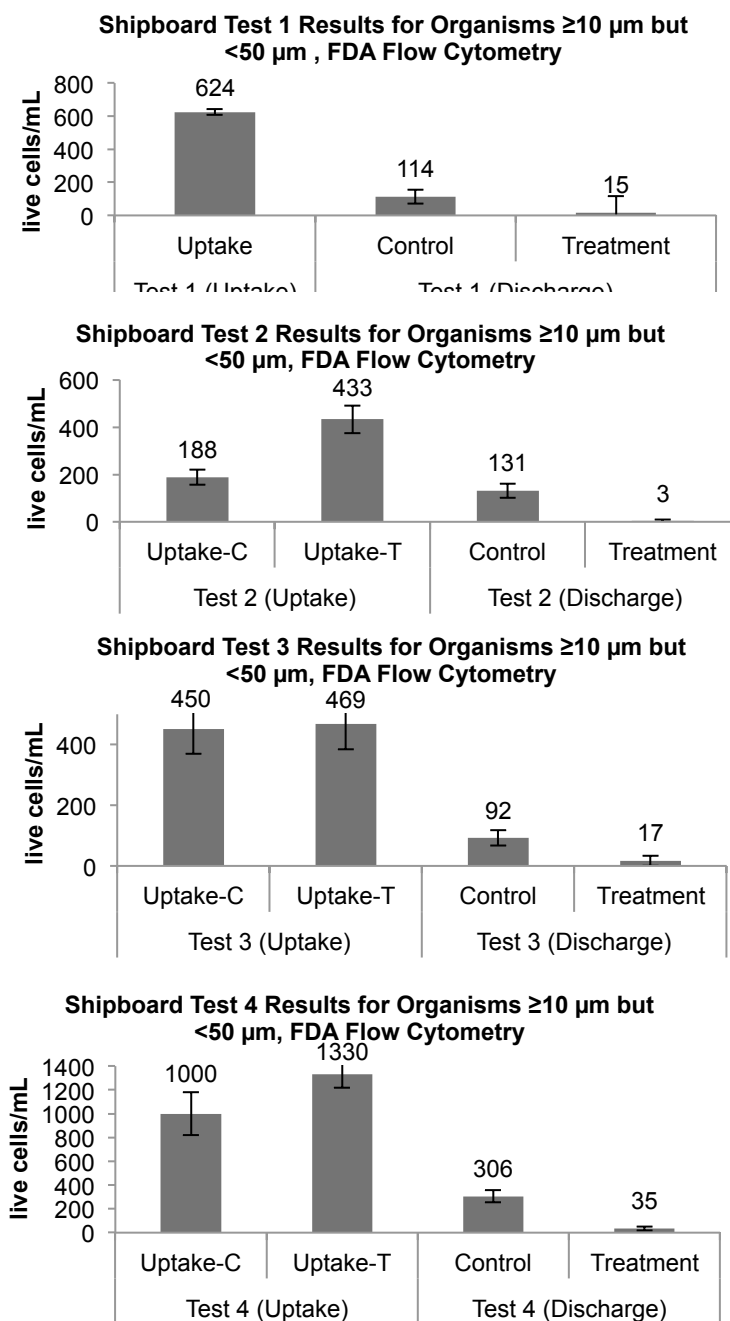


Table 8 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, C-14 Primary Production Results

Test Event	Sample Type	Sample Name	Mean ($\mu\text{g C}/(\text{L}\cdot\text{day})$)	S.D. ($\mu\text{g C}/(\text{L}\cdot\text{day})$)	C.V. (%)	n
1 (Uptake)	Uptake	1U	215.27	3.29	1.53	3
1 (Discharge)	Treatment	1T	1.31	0.24	18.21	3
	Control	1C	45.48	11.59	25.49	3
2 (Uptake)	Treatment	5UT	615.51	95.36	15.49	3
	Control	5UC	396.32	38.76	9.78	3
2 (Discharge)	Treatment	5TD	0.93	0.18	19.16	3
	Control	5CD	112.29	8.22	7.32	3
3 (Uptake)	Treatment	10UT	75.06	4.95	6.60	3
	Control	10UC	63.58	11.93	18.76	3
3 (Discharge)	Treatment	10TD	0.35	0.04	12.94	3
	Control	10CD	10.44	2.98	28.51	3
4 (Uptake)	Treatment	19UT	27.20	3.05	11.23	3
	Control	19UC	28.94	2.07	7.14	3
4 (Discharge)	Treatment	19TD	4.76	0.69	14.56	3
	Control	19CD	14.27	1.10	7.73	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples

Figure 15 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, C-14 Primary Production Results Comparison (right)

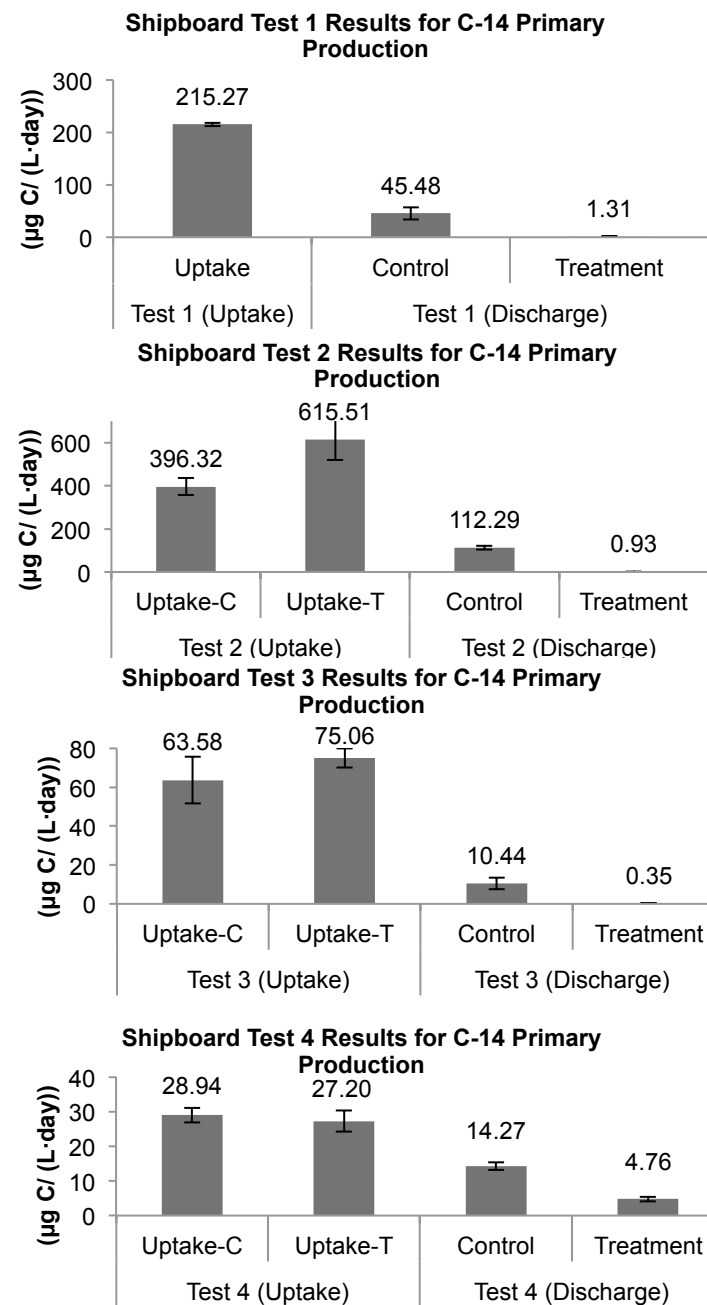
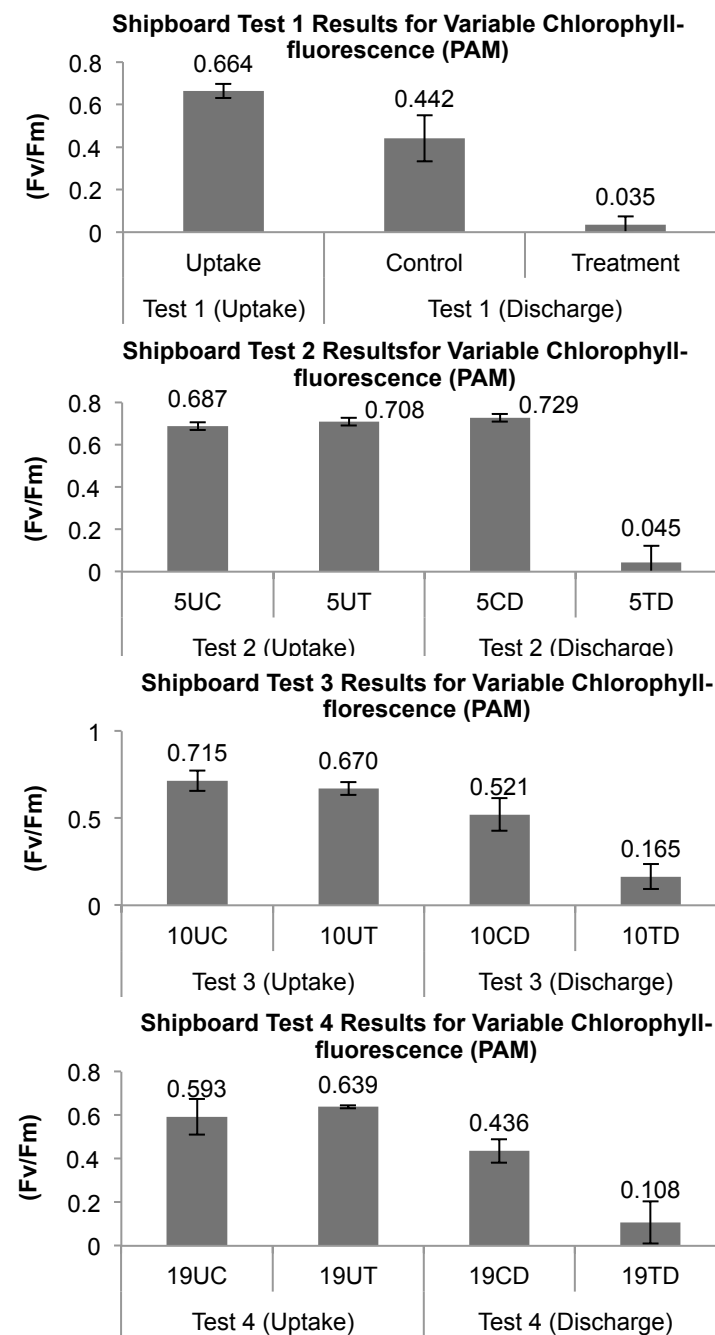


Table 9 - Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$, Variable Chlorophyll-fluorescence (PAM) Results

Test Event	Sample Type	Sample Name	Mean (Fv/Fm)	S.D. (Fv/Fm)	C.V. (%)	n
1 (Uptake)	Uptake	1U	0.664	0.034	5.13	3
1 (Discharge)	Treatment	1T	0.035	0.039	110.06	3
	Control	1C	0.442	0.108	24.56	3
2 (Uptake)	Treatment	5UT	0.708	0.018	2.55	3
	Control	5UC	0.687	0.018	2.61	3
2 (Discharge)	Treatment	5TD	0.045	0.077	173.21	3
	Control	5CD	0.729	0.019	2.58	3
3 (Uptake)	Treatment	10UT	0.670	0.037	5.49	3
	Control	10UC	0.715	0.057	8.03	3
3 (Discharge)	Treatment	10TD	0.165	0.073	44.13	3
	Control	10CD	0.521	0.094	17.96	3
4 (Uptake)	Treatment	19UT	0.639	0.006	1.00	3
	Control	19UC	0.535	0.081	13.72	3
4 (Discharge)	Treatment	19TD	0.108	0.097	90.03	3
	Control	19CD	0.436	0.054	12.43	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples; n/a = not analyzed

Figure 16 - Variable Chlorophyll-fluorescence (PAM) Results Comparison (right)



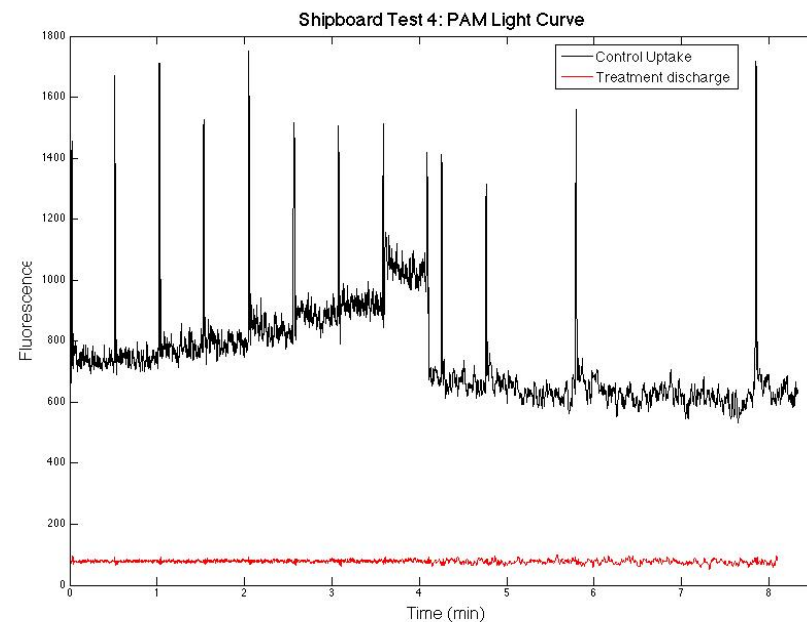
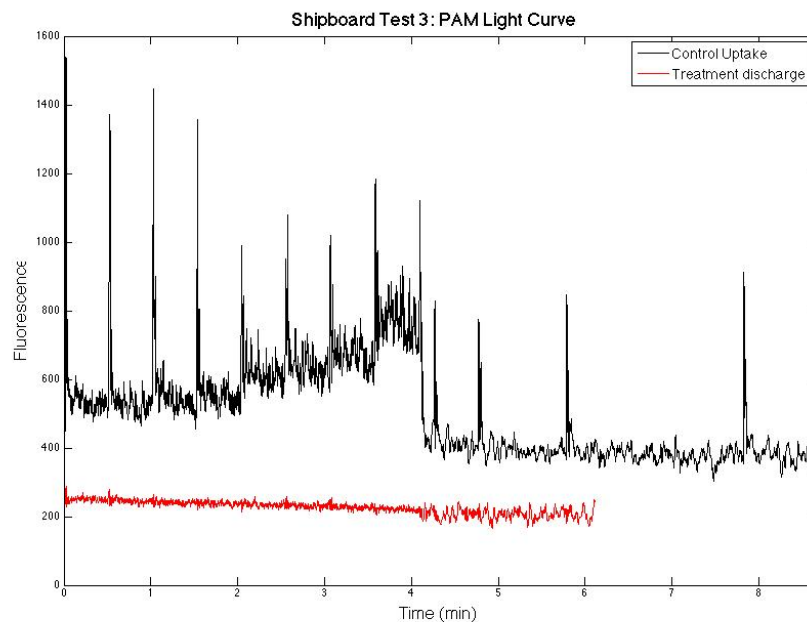
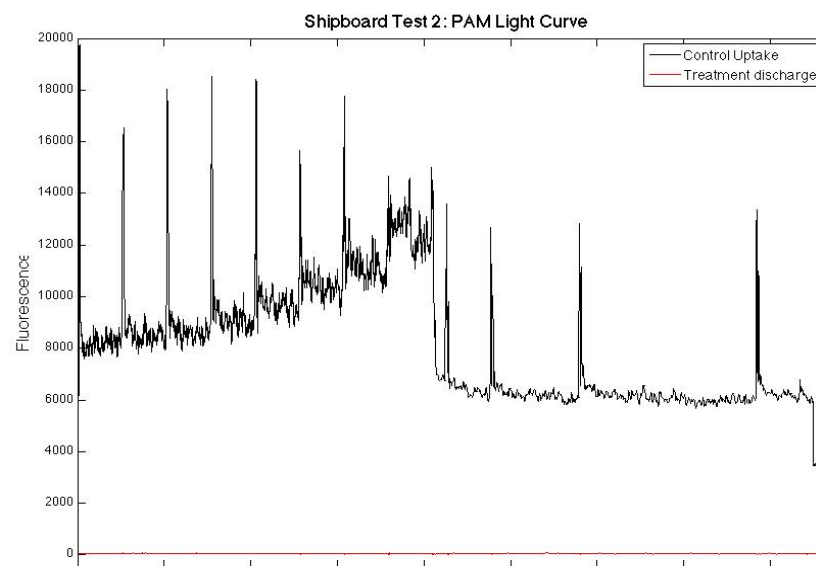
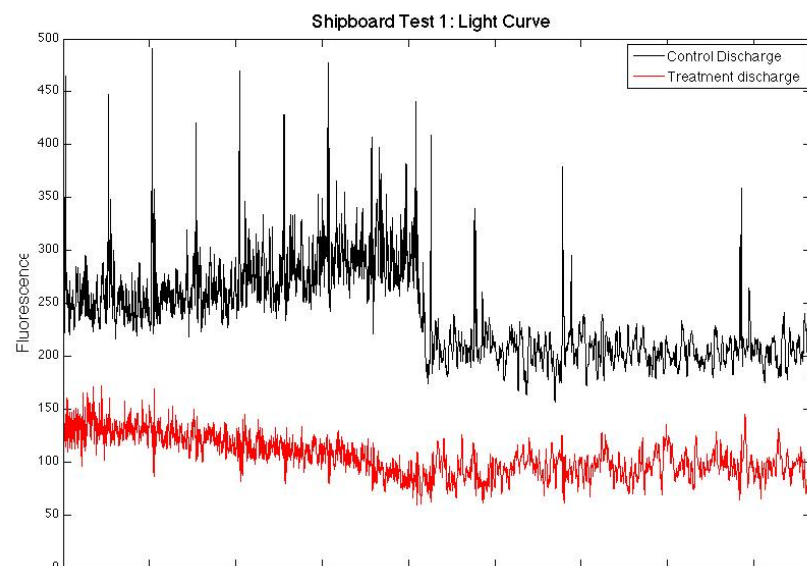


Figure 17 - Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$, Variable Chlorophyll-fluorescence (PAM) Light Curves

Table 10 - Organisms $\geq 10 \mu\text{m}$, Adenosine Triphosphate (ATP) Results

Test Event	Sample Type	Sample Name	Mean (ng/L)	S.D. (ng/L)	C.V. (%)	n
1 (Uptake)	Uptake	1U	n/a	n/a	n/a	n/a
1 (Discharge)	Control	1T	n/a	n/a	n/a	n/a
	Treatment	1T	n/a	n/a	n/a	n/a
2 (Uptake)	Treatment	5UC	n/a	n/a	n/a	n/a
	Control	5UT	n/a	n/a	n/a	n/a
2 (Discharge)	Treatment	5CD	n/a	n/a	n/a	n/a
	Control	5TD	n/a	n/a	n/a	n/a
3 (Uptake)	Treatment	10UT	110.59	14.61	13.21	3
	Control	10UC	105.77	24.91	23.55	3
3 (Discharge)	Treatment	10TD	0.07	0.04	57.02	2
	Control	10CD	18.19	10.85	59.63	3
4 (Uptake)	Treatment	19UT	158.99	34.38	21.62	3
	Control	19UC	42.72	19.14	44.82	3
4 (Discharge)	Treatment	19TD	1.52	0.30	19.60	3
	Control	19CD	21.17	14.39	67.94	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples;
n/a = not analyzed

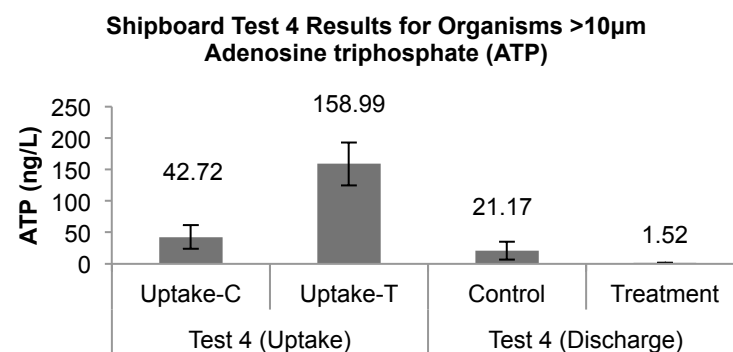
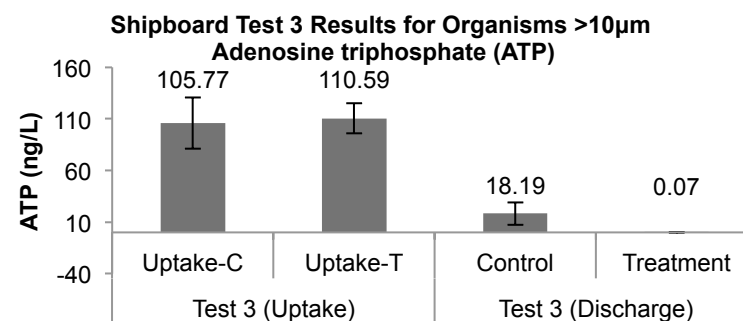


Figure 18 - Organisms $\geq 10 \mu\text{m}$, Adenosine Triphosphate (ATP) Results Comparison (above)

8.1.3 Indicator Microbes <10 µm

Escherichia coli and *Enterococci* by Most Probable Number (MPN). Tables 11-12 and Figures 19-20 show results for numeric colony forming units determined for *E. coli* and *Enterococci* using Idexx 24 hour incubation kits at 35 °C. Typical of natural waters, *E. coli* counts were low (<20 CFU/100 mL) for all samples from all shipboard Tests 1, 2, 3 and 4. Thus, even the untreated water met the IMO D-2 standard of <250 CFU/100 mL. It may be of some significance to note, however, that all *treatment* discharge samples showed no positive chromogenic scores in any one of the 51 MPN counting cells on the Idexx counting trays; those results were reported as zero. In contrast, at least one positive growth well was detected in all *untreated* samples (uptake and discharge) providing some evidence that UV inactivation was evident in treatment discharge (see Table 11).

Enterococci counts were significantly higher than for *E. coli* in all untreated samples, at times exceeding 200.5 CFU/100 mL, the upper detection limit for the Idexx counting kit(see Tables 11-12). In contrast, average *Enterococci* counts from all *treatment* discharge samples were ≤1 CFU/100 mL. Thus, UV inactivation provided at least a 100-fold reduction in ambient *Enterococci* concentrations, easily meeting the IMO D-2 standard of <100 CFU/100 mL.

Vibrio cholerae, Serotypes 01 and 0139. Tests for *V. cholerae* serotypes 01 and 0139 were conducted for all uptake control and treatment discharge samples during shipboard Tests 1, 2, 3 and 4. All tests showed no detectable levels of *V. cholerae*, <1 CFU/100 mL (data not shown). Thus, the treatment discharge met the IMO D-2 discharge standard. However, no useful information was gathered regarding BWMS biological efficacy since the ambient challenge concentrations were also undetectable.

Heterotrophic Plate Counts (HPC) (Corroborative). Results for HPC are given in Table 13 and Figure 21. Final HPC levels in treatment discharge from all four shipboard tests showed reductions relative to the control uptake; the reduction ranged from two-fold to seven-fold, approximately. A regulatory limit for tolerable ballast water HPC levels does not exist for IMO nor United States Coast Guard (USCG) regulations. However, United States Environmental Protection Agency (USEPA) ETV land-based testing guidelines (ETV Protocol, 2010) demand that control discharge ballast tanks yield a final challenge concentration $>5 \times 10^2$ CFU/mL; a condition that was met in all four shipboard tests (see Table 13). All treatment discharge samples reported here were lower than the challenge control concentration above, thus confirming measurable HPC reduction by the tested BWMS.

Table 11 - Indicator Microbes <10 µm, *E. coli* Results

Test Event	Sample Type	Sample Name	Mean (CFU/100mL)	S.D. (CFU/100mL)	C.V. (%)	n
1 (Uptake)	Uptake	1U	0.22	0.44	198	9
1 (Discharge)	Treatment	1T	0	0	0	9
	Control	1C	0	0	0	9
2 (Uptake)	Treatment	5UT	1.70	1.57	92.45	3
	Control	5UC	0.67	1.15	173.21	3
2 (Discharge)	Treatment	5TD	0	0	0	3
	Control	5CD	0.33	0.58	173	3
3 (Uptake)	Treatment	10UT	1.00	1.00	100	3
	Control	10UC	3.50	2.72	78	3
3 (Discharge)	Treatment	10TD	0	0	0	3
	Control	10CD	16.03	4.25	26	3
4 (Uptake)	Treatment	19UT	0.67	0.58	87	3
	Control	19UC	2.37	0.64	27	3
4 (Discharge)	Treatment	19TD	0	0	0	3
	Control	19CD	0.33	0.58	173	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples

Figure 19 - Indicator Microbes <10 µm, *E. coli* Results Comparison (right)

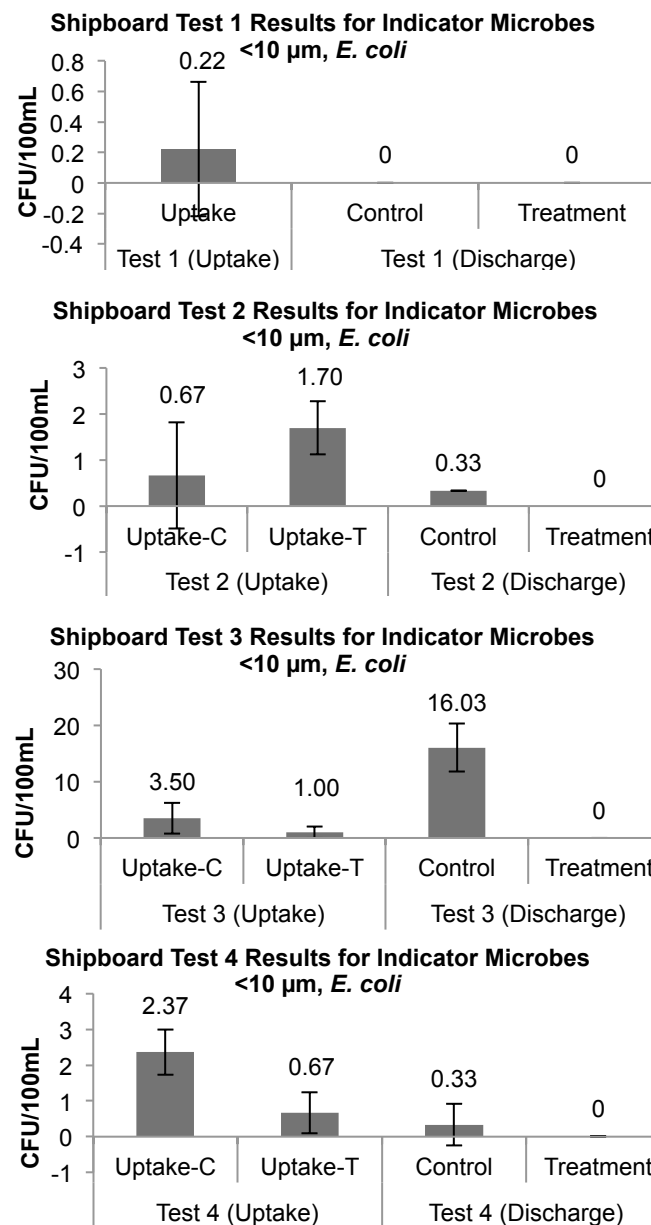


Table 12 - Indicator Microbes <10 µm, *Enterococci* Results

Test Event	Sample Type	Sample Name	Mean (CFU/100mL)	S.D. (CFU/100mL)	C.V. (%)	n
1 (Uptake)	Uptake	1U	181.40	31.30	17	9
1 (Discharge)	Treatment	1T	0.13	0.35	283	9
	Control	1C	109.41	29.19	27	9
2 (Uptake)	Treatment	5UT	75.73	8.86	12	3
	Control	5UC	21.80	3.46	16	3
2 (Discharge)	Treatment	5TD	0	0	0	3
	Control	5CD	16.77	7.90	47	3
3 (Uptake)	Treatment	10UT	98.67	26.96	27	3
	Control	10UC	134.73	36.35	27	3
3 (Discharge)	Treatment	10TD	0.33	0.58	173	3
	Control	10CD	83.60	8.49	10	3
4 (Uptake)	Treatment	19UT	146.47	47.93	33	3
	Control	19UC	200.50	0.00	0	3
4 (Discharge)	Treatment	19TD	1.00	1.00	100	3
	Control	19CD	200.50	0.00	0	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples

Figure 20 - Indicator Microbes <10 µm, *Enterococci* Results Comparison (right)

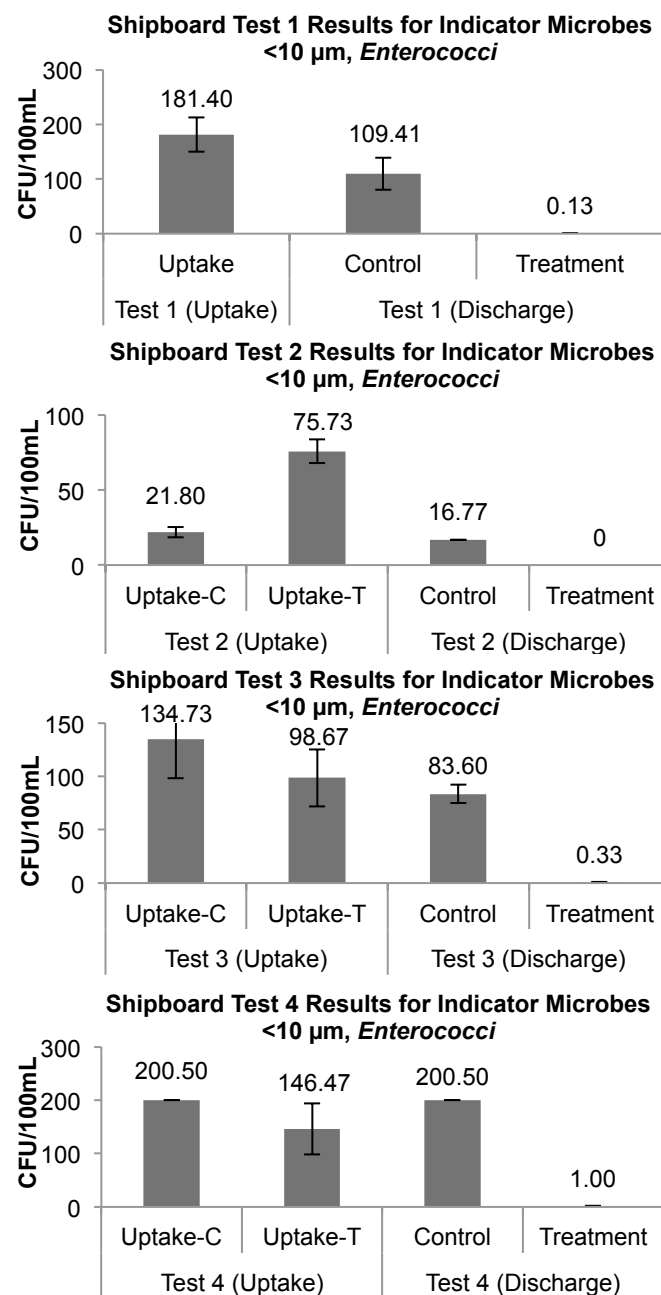
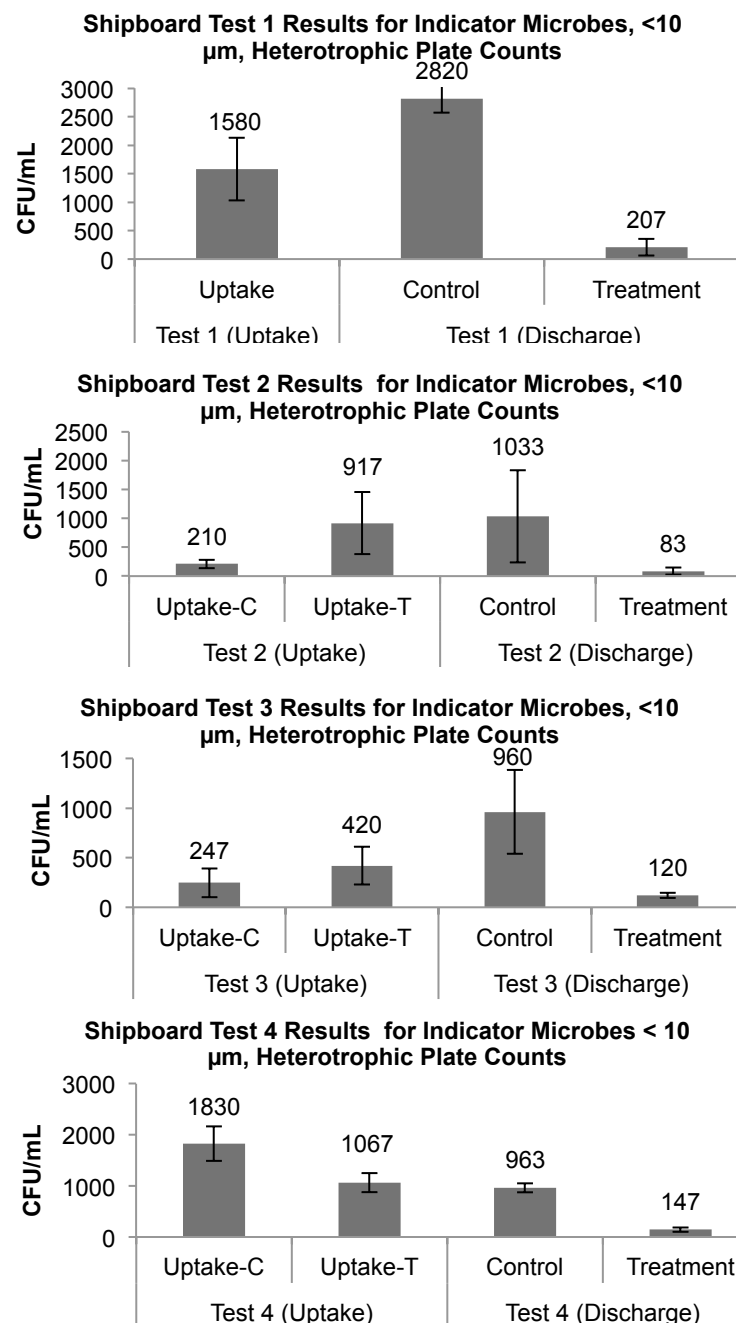


Table 13 - Indicator Microbes <10 µm, Heterotrophic Plate Count Results

Test Event	Sample Type	Sample Name	Mean (CFU/mL)	S.D. (CFU/mL)	C.V. (%)	n
1 (Uptake)	Uptake	1U	1580	550	35	3
1 (Discharge)	Treatment	1T	207	145	70	3
	Control	1C	2820	250	9	3
2 (Uptake)	Treatment	5UT	917	535	58	3
	Control	5UC	210	75	36	3
2 (Discharge)	Treatment	5TD	83	61	73	3
	Control	5CD	1033	801	78	3
3 (Uptake)	Treatment	10UT	420	192	46	3
	Control	10UC	247	145	59	3
3 (Discharge)	Treatment	10TD	120	26	22	3
	Control	10CD	960	423	44	3
4 (Uptake)	Treatment	19UT	1067	188	18	3
	Control	19UC	1830	339	19	3
4 (Discharge)	Treatment	19TD	147	45	31	3
	Control	19CD	963	92	10	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples

Figure 21 - Indicator Microbes <10 µm, Heterotrophic Plate Count Results Comparison (right)



8.2 Water Quality

Chemical and physical water quality parameters are summarized in Tables 14-20 below. Generally, the turbid San Francisco Bay estuarine conditions provided brackish salinities (16-32 PSU) and high sediment loads (45-65 mg/L TSS). There are no IMO test validity criteria for challenge water during shipboard testing, but the brackish water of San Francisco Bay provided TSS loads that met IMO land-based test validity criteria on two of the four shipboard tests conducted. In all four tests, the Chlorophyll α concentration decreased in both treatment and control discharge samples relative to uptake samples, suggesting a loss of algal biomass during the five day holding period.

Table 14 - Continuous In-Situ and In-line Water Quality Results

Test Event	Sample Type	Temperature In-situ (°C)	Temperature In-line (°C)	Salinity Range In-situ (PSU)	Dissolved Oxygen In-situ (mg/L)
1 (Uptake)	Uptake	11.56	13.0	29.09-29.43	8.97
1 (Discharge)	Treatment	12.84	14.1	28.7-29.34	9.05
	Control	14.58	14.1	26.61-27.14	8.79
2 (Uptake)	Treatment	13.35	13.8	16.38-30.49	8.89
	Control	10.22	11.1	27.47-33.13	7.69
2 (Discharge)	Treatment	17.18	18.3	29.40-29.59	8.60
	Control	17.35	18.2	30.42-32.84	8.30
3 (Uptake)	Treatment	16.74	18.0	19.69-31.59	7.85
	Control	16.52	17.7	31.8-31.8	7.63
3 (Discharge)	Treatment	n/a	19.4	n/a	n/a
	Control	n/a	19.3	n/a	n/a
4 (Uptake)	Treatment	13.21	14.5	28.36-28.52	8.64
	Control	13.12	14.4	26.90-28.37	8.52
4 (Discharge)	Treatment	14.48	15.7	27.31-28.32	8.42
	Control	14.70	15.5	25.77-25.94	8.94

n/a = not analyzed

Table 15 - POC Results

Test Event	Sample Type	Sample Name	Mean (mg/L)	S.D. (mg/L)	C.V. (%)	n
1 (Uptake)	Uptake	1U	0.84	0.02	1.97	3
1 (Discharge)	Treatment	1T	0.37	0.02	4.23	3
	Control	1C	0.26	0.03	10.78	3
2 (Uptake)	Treatment	5UT	0.45	0.02	3.64	3
	Control	5UC	0.48	0.01	1.74	3
2 (Discharge)	Treatment	5TD	0.47	0.02	3.34	3
	Control	5CD	0.55	0.02	4.26	3
3 (Uptake)	Treatment	10UT	0.56	n/a	n/a	1
	Control	10UC	0.45	n/a	n/a	1
3 (Discharge)	Treatment	10TD	0.46	n/a	n/a	1
	Control	10CD	0.39	n/a	n/a	1
4 (Uptake)	Treatment	19UT	0.59	0.16	28.16	3
	Control	19UC	1.56	0.18	11.80	3
4 (Discharge)	Treatment	19TD	0.30	0.03	11.73	3
	Control	19CD	0.59	0.05	7.62	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples; n/a = not analyzed

Table 16 - DOC Results

Test Event	Sample Type	Sample Name	Mean (mg/L)	S.D. (mg/L)	C.V. (%)	n
1 (Uptake)	Control	1U	1.9	0.06	3.1	3
1 (Discharge)	Control	1CD	n/a	n/a	n/a	n/a
	Treatment	1TD	n/a	n/a	n/a	n/a
2 (Uptake)	Control	5UC	1.5	0.47	32.5	3
	Control	5UT	1.8	0.15	8.6	3
2 (Discharge)	Control	5CD	n/a	n/a	n/a	n/a
	Treatment	5TD	n/a	n/a	n/a	n/a
3 (Uptake)	Control	10UC	2.2	n/a	n/a	1
	Control	10UT	1.9	n/a	n/a	1
3 (Discharge)	Control	10CD	n/a	n/a	n/a	n/a
	Treatment	10TD	n/a	n/a	n/a	n/a
4 (Uptake)	Control	19UC	2.4	n/a	n/a	1
	Control	19UT	2.4	n/a	n/a	1
4 (Discharge)	Control	19CD	n/a	n/a	n/a	n/a
	Treatment	19TD	n/a	n/a	n/a	n/a

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples; n/a = not analyzed

Table 17 - TSS Results

Test Event	Sample Type	Sample Name	Mean (mg/L)	S.D. (mg/L)	C.V. (%)	n
1 (Uptake)	Uptake	1U	59.37	1.69	2.84	3
1 (Discharge)	Treatment	1T	47.71	2.93	6.15	3
	Control	1C	40.47	2.39	5.91	3
2 (Uptake)	Treatment	5UT	32.51	3.35	10.30	3
	Control	5UC	26.14	0.04	0.17	3
2 (Discharge)	Treatment	5TD	14.68	0.95	6.47	3
	Control	5CD	17.40	1.06	6.07	3
3 (Uptake)	Treatment	10UT	29.27	1.65	5.65	3
	Control	10UC	23.14	3.58	15.47	3
3 (Discharge)	Treatment	10TD	27.37	0.82	2.98	3
	Control	10CD	24.31	0.18	0.76	3
4 (Uptake)	Treatment	19UT	40.65	2.22	5.47	3
	Control	19UC	105.37	3.36	3.19	3
4 (Discharge)	Treatment	19TD	39.40	1.71	4.33	3
	Control	19CD	42.68	5.48	12.84	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples; n/a = not analyzed

Table 18 - Chlorophyll α Results

Test Event	Sample Type	Sample Name	Mean Chl α ($\mu\text{g/L}$)	S.D. ($\mu\text{g/L}$)	C.V. (%)	n
1 (Uptake)	Uptake	1U	2.26	0.54	24	3
1 (Discharge)	Treatment	1T	0.66	0.12	18	3
	Control	1C	0.56	0.15	27	3
2 (Uptake)	Treatment	5UT	9.21	0.42	5	3
	Control	5UC	8.44	1.11	13	3
2 (Discharge)	Treatment	5TD	0.64	0.03	5	3
	Control	5CD	1.73	0.14	8	3
3 (Uptake)	Treatment	10UT	3.18	0.25	8	3
	Control	10UC	2.49	0.28	11	3
3 (Discharge)	Treatment	10TD	1.07	0.11	10	3
	Control	10CD	0.66	0.04	6	3
4 (Uptake)	Treatment	19UT	5.38	0.38	7	3
	Control	19UC	4.79	0.33	7	3
4 (Discharge)	Treatment	19TD	0.95	0.15	16	3
	Control	19CD	0.77	0.09	11	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples

Table 19 - pH Results

Test Event	Sample Type	Sample Name	Mean (pH)	S.D. (pH)	C.V. (%)	n
1 (Uptake)	Uptake	1U	7.987	0.01	0.07	3
1 (Discharge)	Treatment	1T	7.923	0.00	0.03	3
	Control	1C	7.930	0.01	0.09	3
2 (Uptake)	Treatment	5UT	8.002	0.01	0.18	3
	Control	5UC	7.894	0.01	0.17	3
2 (Discharge)	Treatment	5TD	8.000	0.01	0.17	3
	Control	5CD	7.937	0.03	0.40	3
3 (Uptake)	Treatment	10UT	7.917	0.01	0.12	3
	Control	10UC	7.942	0.00	0.01	3
3 (Discharge)	Treatment	10TD	7.907	0.01	0.15	3
	Control	10CD	7.875	0.04	0.51	3
4 (Uptake)	Treatment	19UT	7.941	0.01	0.10	3
	Control	19UC	7.949	0.00	0.05	3
4 (Discharge)	Treatment	19TD	7.927	0.01	0.16	3
	Control	19CD	7.884	0.00	0.05	3

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples

Table 20 - UV Transmittance Results

Test Event	Sample Type	Sample Name	Mean (%)	S.D. (%)	C.V. (%)	n
1 (Uptake)	Uptake	1U	78.78	1.90	2.41	3
1 (Discharge)	Treatment	1T	n/a	n/a	n/a	n/a
	Control	1C	n/a	n/a	n/a	n/a
2 (Uptake)	Treatment	5UT	90.71	0.64	0.70	3
	Control	5UC	95.80	1.41	1.47	3
2 (Discharge)	Treatment	5TD	n/a	n/a	n/a	n/a
	Control	5CD	n/a	n/a	n/a	n/a
3 (Uptake)	Treatment	10UT	87.5	3.24	3.7	12
	Control	10UC	87.49	3.24	3.70	9
3 (Discharge)	Treatment	10TD	87.8	1.54	1.75	12
	Control	10CD	87.80	1.54	1.76	12
4 (Uptake)	Treatment	19UT	93.04	0.61	1	3
	Control	19UC	80.55	3.78	5	3
4 (Discharge)	Treatment	19TD	n/a	n/a	n/a	n/a
	Control	19CD	n/a	n/a	n/a	n/a

S.D. = standard deviation; C.V. = coefficient of variation; n = number of subsamples; n/a = not analyzed

9 DISCUSSION AND CONCLUSION

IMO D-2 standards for viable organism concentrations in shipboard testing include the following concentrations: 1) <10 viable organisms/m³ for organisms ≥50 µm in minimum dimension, 2) <10 viable organisms/mL for organisms ≥10 µm but <50 µm in minimum dimension, 3) <250 CFU/100 mL of *E. coli*, 4) <100 CFU/100 mL of *Enterococci* and 5) <1 CFU/100 mL of *V. cholerae* serotypes 01 and 0139. All numeric methods approved for viability testing described in the GBF TQAP yielded results that met IMO D-2 standards (see Section 8).

In several cases, the measured viable organism concentrations were considerably lower than specified by IMO D-2 standards. For instance, the combined measurements of organisms ≥50 µm (zooplankton) yielded a total of 37 viable organisms in a total processed ballast water volume of 40 m³, or 0.93 viable organisms/m³; this value is more than 10 times below the IMO D-2 discharge standard. Moreover, the data met statistical requirements for counts and representative sampling volumes articulated by Miller et al. (2011) in order to constitute valid regulatory concentration evaluations. It appeared that the Trojan Marinex™ BWT 250 achieved efficient reduction of organisms ≥50 µm largely as a result of filtration alone, as evidenced by concentrations of 'dead' organisms that ranged from only 20-100 organisms/m³ in treatment discharge samples compared to more than 30,000 organisms/m³ in ambient uptake water (see Table 6). That is, the bulk of organism reduction/inactivation in the largest size class was affected by filtration, not UV inactivation alone.

The observed treatment discharge concentrations for viable organisms ≥10 µm but <50 µm based on MPN measurement yielded an average concentration of approximately 0.055 viable organisms/mL, more than two orders of magnitude lower than specified by IMO D-2 standards.

Enterococci concentrations were two orders of magnitude lower than the IMO D-2 standard. No detectable *E. coli* were found in any of the 18 treatment discharge samples processed in these shipboard tests (see Table 11). However, the efficacy implied by the results for *E. coli* must be tempered by the realization that *E. coli* concentrations were also extremely low (maximum 20 CFU/100 mL) in all untreated control samples. *V. cholerae* was also absent from treatment discharge samples but no meaningful BWMS efficacy is implied here because *V. cholera* was not detected in any sample analyzed, treated or untreated.

Corroborative assays represented by 1) cell-specific FDA viability tagging (flow cytometry) 2) C-14 based photosynthesis, 3) PAM-based variable chlorophyll-fluorescence, 3) ATP, and 5) HPCs all showed dramatic reductions in measurement signals for treated discharge samples relative to controls.

The numeric counts of viable organisms ≥10 µm but <50 µm generated from cell-specific FDA tagging with flow cytometric enumeration showed large reductions in cell counts (approximately 40-fold), but final treatment concentrations did not meet the IMO D-2 standard in three of the four shipboard tests (see Table 7 and Figure 14). Given the evidence from the corroborative measurements above, especially those showing more than 100-fold reductions, and given the significant contrast in numeric results relative to MPN grow out incubations monitored for 2-6 weeks (see

Table 6 and Figures 12-13), it appears that the problem of FDA cell-specific false positives from UV treatment cited by others was also in effect during shipboard testing.

The ability of cell-specific FDA viability tagging to unambiguously mark 'live' cells after UV treatment is currently under discussion. Previous research has indicated false-positives from FDA-based cell-specific fluorescence analysis (Steinberg et al., 2011). That is, FDA-marked live (green) cells have been detected in samples known to contain true dead cells (Drake et al., 2010). The false-positive problem is more acute for UV-induced inactivation, since UV treatment may not render the cell dead per se, but presumably DNA-damaged and thus incapable of reproduction. Remnant enzyme activity, capable of FDA hydrolysis to fluorescein, would be a distinct possibility for intact cells suffering DNA-damage but otherwise retaining some internal biochemical integrity. For this reason, the absolute value of the live numeric cell concentration derived from FDA-marked cells should be judged cautiously, and corroborated by alternative methods (see below).

C-14 based photosynthetic rates and ATP concentrations were reduced by more than two orders of magnitude (see Tables 8 and 10 and Figures 15 and 18). PAM fluorescence traces during rapid light-curve experiments showed dramatic and permanent reduction of signals for Fv/Fm in treatment samples that rendered the potential biological signal difficult to discern within the noise of the background signal (see Figure 17).

HPCs were reduced by over one order of magnitude from uptake to treatment discharge in three of the four shipboard tests (see Table 13). HPCs provide corroborative data on bacterial responses to ballast water treatment that otherwise may not be evident for the IMO species-specific assays for indicator microbes. Some indicator species may be completely absent in uptake water, thus providing no useful data for evaluation of BWMS biological efficacy, as evidenced by assays for *V. cholerae* during shipboard testing of the Trojan Marinex™ BWT 250. Generally, a broad spectrum of cultivable bacteria species is present in all natural waters providing measurable HPC scores (CFU/mL) in all ballasting events. Typical ambient bacteria concentrations given by cultivable HPCs (10^2 - 10^3 /mL) are approximately 1000-fold lower than total direct bacteria counts (10^5 - 10^6 /mL). That is, the cultivable bacteria fraction (HPC) constitutes a small fraction of the *total* bacterial concentration. However, HPC concentrations are typically two to four orders of magnitude greater than any IMO individual indicator species, thus providing more meaningful information on treatment effects for a given BWMS. Some caution should be exercised in formulating expectations for BWMS treatment efficacy on the basis of HPCs (e.g., no detectable HPC under effective treatment) since non-sterile conditions onboard ship, and especially within the ballast pipe/tank system, make total elimination of HPC highly unlikely. During shipboard testing of the Trojan Marinex™ BWT 250, a two-fold to seven-fold reduction in HPCs was evident from UV treatment.

The general results from all four shipboard tests conducted to evaluate the Trojan Marinex™ BWT 250 biological efficacy were compliant with all IMO D-2 standards. The final treatment discharge results of all required numerical biological tests were reduced one to two orders of magnitude more than required by IMO D-2 standard. All corroborative assays conducted in addition to required IMO numeric testing also supported the findings above.

10 ACKNOWLEDGEMENTS

The development and operation of the Cal Maritime, Golden Bear Facility was made possible by the support of the US Maritime Administration, California State Lands Commission, NOAA SeaGrant program, University of Washington Department of Fisheries, American Bureau of Shipping, ITT Process and Control, California Maritime Academy (CMA), Moss Landing Marine Laboratories (MLML), and The Glosten Associates.

Preparation, testing, and reporting would not have been possible without the efforts of personnel at CMA, MLML, and others. The staff, faculty and crew of the USTS *Golden Bear* of CMA that conducted this project include:

- Bill Davidson (Facility Director);
- Richard Muller (Test Manager and Quality Officer);
- Nic Shields (Primary Ballast Operator and 3rd Mate CMA);
- Harry Bolton (Captain CMA);
- John Coyle (First Engineer and Relief Chief Engineer CMA);
- Matt Hobbs (3rd Engineer CMA);
- James Eckerle (3rd Engineer CMA);
- Dan Lintz (Chief Mate CMA);
- Bill Schmid (Relief Chief Mate CMA);
- Dave Coleman (2nd Mate CMA); and
- Mick Bowlin (Chief Electrician CMA).

The scientific staff from MLML that performed the testing, analysis, and reporting includes:

- Dr. Nicholas Welschmeyer, Ph.D (Lead Scientist);
- Brian Maurer (BS Biology, Masters Marine Science, MLML);
- Nicole Bobco (BS Biology, MLML Masters candidate);
- Jules Kuo (BS Biology, MLML Masters candidate);
- Heather Fulton-Bennett (BS Biology, MLML Masters candidate; backup assistant);
- Catherine Drake (BS Biology, MLML Masters candidate, MLML)
- Marilynn Koopnick (BS Biology; Masters Marine Science, MLML);
- Michelle Marraffino (BS Biology; Masters candidate, MLML);
- Kristin Meagher (BS Biology; Masters candidate, MLML); and
- Elizabeth Lam (BS Biology, UC Berkeley).

Appendix A Test Quality Assurance Plan Red-line

Test Quality Assurance Plan

Project Charlie

Golden Bear Facility
Vallejo, California

Dept. 76408, Fund 46408
9 April 2012, Rev. A

Red-line 28 August, 2012

QAPP (Land-Based): Pages 21, 23, 24, 26, and 27

QAPP (Shipboard): Pages 20, and 23

SOPs:

SOP 2 pages 12 & piping line-ups sheet 2-13

SOP 3 piping line-ups sheet 6&7

SOP 8 page 49

SOP 10 pages 57 & 68

SOP 14 pages 76, 80-104

SOP 15 page 126

SOP 16 pages 135-138, 141, 146, and 150

Red-line 16 March, 2013

(Shipboard filtration on Uptake only)

QAPP (Shipboard): Land-based test cancelled

QAPP (Shipboard): Pages 20, and 23

SOPs:

SOP 3 piping line-ups sheet 6&7

SOP 15 page 126

Test Quality Assurance Plan

Project Charlie

Golden Bear Facility
Vallejo, California

Dept. 76408, Fund 46408
9 April 2012, Rev. A

BY: _____

Nicholas Welschmeyer, PhD

Lead Investigator
Golden Bear Facility

CHECKED: _____

Richard Muller

Quality Officer
Golden Bear Facility

APPROVED: _____

William T. Davidson

Facility Director
Golden Bear Facility

ACCEPTED: _____

Verification Organization
Det Norske Veritas

ACCEPTED: _____

Client
Trojan Marinex



Preface

This Test Quality Assurance Plan (TQAP) is specific to testing of the Project Charlie ballast water treatment system (BWTS) at the Golden Bear Facility (Facility). This project has been commissioned to determine if this BWTS meets international standards for Land-Based brackish and marine water conditions, and Shipboard conditions.

Test Documentation

With a focus on quality control and quality assurance, the following volumes make up the Test Quality Assurance Plan that is submitted to the Verification Organization (VO). The Facility's documentation control process is detailed in the standard operating procedures of the Quality Management Plan (QMP).

Test Quality Assurance Plan (TQAP) – Project Charlie		
I	Project Plan	Project specific plan that identifies the testing objectives and conditions, describes the testing facility and equipment to be evaluated, and establishes the testing logistics, including personnel and calendar dates.
II	Quality Assurance Project Plan (Land-Based)	Quality assurance/quality control measures are established for executing the SOPs to test the treatment system under Land-Based conditions. In addition, the protocols for evaluating test biological and chemical conditions are established.
III	Quality Assurance Project Plan (Shipboard)	Quality assurance/quality control measures are established for executing the SOPs to test the treatment system under Shipboard conditions. In addition, the protocols for evaluating test biological and chemical conditions are established.
IV	Standard Operating Procedures (SOPs)	SOPs for operations and sampling are given that are specific to this testing.
V	BWTS Operation, Maintenance, & Installation Manual	Manufacturer information for the equipment being tested.

The Test Quality Assurance Plan (TQAP) consists of Project Charlie specific Project Plan (Plan), Land-Based Quality Assurance Project Plan (QAPP), Shipboard QAPP, and Standard Operating Procedures (SOPs). These documents reference the Facility Quality Management Plan (QMP). Wherever a conflict exists between documents, the SOPs takes first precedence and the QAPP takes second precedence.

Test Quality Assurance Plan – Project Charlie

Volume I: Project Plan

Golden Bear Facility
Vallejo, California

Dept. 76408, Fund 46408
9 April 2012, Rev. A



Contents

Section 1 Summary	1
Section 2 Facility Description	2
2.1 Overview	2
Section 3 Ballast Water Treatment System (BWTS)	3
3.1 Technology Description	3
3.2 Scope of Supply.....	6
3.3 BWTS Monitoring and Documentation	6
Section 4 Organization	8
4.1 Project Roles and Responsibilities	8
Section 5 Project Objective and Description	10
5.1 Project Objective	10
5.2 Project Description	10
5.3 Nomenclature	10
5.4 Testing Sequence.....	11
5.5 Project Schedule	11
5.6 Documentation	14
5.7 Verification Organization Inspection/Audit	15
5.8 Biological Efficacy Testing.....	15
Section 6 Experimental Approach	16
6.1 Quality Control and Quality Assurance.....	16
6.2 Automation System and Online Information System.....	16
6.3 Challenge Water	17
6.4 System Commissioning and Tank Flushing	19
6.5 Biological Efficacy Testing and Valid Test Parameters.....	19
6.6 Test Validation Review (Failures).....	23
6.7 Decommissioning and Reporting	24
Section 7 Environmental, Health & Safety Plan	25
7.1 GBF EH&S Plan	25
7.2 References	25
7.3 Hazardous Materials	25
7.4 Lithium Batteries	26
7.5 Storage Containers.....	26
7.6 Compressed Gases.....	26

7.7	Spill Response	26
7.8	Sharps Disposal	26
7.9	Responsibility	26
7.10	Contacts—EH&S Response	27

Appendix A: California Maritime Academy EH&S Policy

Appendix B: Contact Information for Key Personnel

References

- 001 *Quality Management Plan*, Golden Bear Facility, December 2011.
- 002 *Environmental, Health, and Safety Plan*, Golden Bear Facility, November 2010.
- 003 *Facility Physical Plant Description*, Golden Bear Facility, November 2010.
- 004 *Quality Assurance Project Plan (Land-Based Testing) – Project Charlie*, Golden Bear Facility, March 2012.
- 005 *Quality Assurance Project Plan (Shipboard Testing) – Project Charlie*, Golden Bear Facility, March 2012.
- 006 *Standard Operating Procedures – Project Charlie*, Golden Bear Facility, March 2012.
- 007 *Protocol for the Verification of Ballast Water Treatment Technologies*, US Environmental Protection Agency, September 2010.
- 008 *Guidelines for Approval of Ballast Water Management Systems (G8)*, Annex 4, MEPC 174(58), Marine Environment Protection Committee, October 2008.

Section 1 Summary

This Project Plan (Plan) is specific to the testing of the Project Charlie ballast water treatment system (BWTS) at the Golden Bear Facility (Facility). This project has been commissioned to determine if this BWTS meets international standards for both **Shipboard** and **Land-Based** testing. Shipboard testing will be conducted with ambient water at various California inland and coastal locations. Land-based testing will be conducted in brackish and marine water conditions. During Land-Based testing, ambient seawater from Carquinez Strait will be augmented with various chemicals and concentrated ambient biological constituents to meet required challenge water conditions.

This Plan identifies the testing objectives and conditions, describes the testing facility and BWTS to be evaluated, and establishes the testing logistics, including personnel and calendar dates. The Plan is one part of the Test Quality Assurance Plan (TQAP) that also consists of Project Charlie specific Shipboard Quality Assurance Project Plan (QAPP), Land-based QAPP, and Standard Operating Procedures (SOPs). This Plan references the Facility Quality Management Plan (QMP). Wherever a conflict exists between documents, the SOPs takes first precedence and the QAPP takes second precedence.

Table 1 - Project Charlie Particulars

Test Organization	Golden Bear Facility, Vallejo, California
Verification Organization	Det Norske Veritas (DNV)
Client	Trojan Technologies, Ontario, Canada.
Device	Ballast Water Treatment System BWTS.
Commissioning	January 2012
Shipboard Testing	Test 1,2,3,4: March, April, June, September, 2012
Land-Based Testing Period	March 2012 through September 2012. At least five (5) tests in brackish water conditions and five (5) tests in marine conditions.
Report	Draft data report three weeks from completion of last test. Full draft report 60 days from completion of last test. Final report following receipt of comments.
Test Parameters	Land-based and Shipboard conditions. Flow rate 250 m ³ /hr +/-10%. Treatment volume 200 – 240 m ³ . Control volume 200 – 220 m ³ .
Test Protocols	Test Protocol. Quality Assurance Project Plans and Standard Operating Procedures – Project Charlie, Golden Bear Facility. Protocol Basis – Required. Guidelines for Approval of Ballast Water Management Systems (G8), MEPC.174(58) Protocol Basis – Supplemental. Generic Protocol for the Verification of Ballast Water Treatment Technology, ETV, September 2010.

Section 2 Facility Description

2.1 Overview

The Golden Bear Facility (GBF) is located on the 500-foot long *Training Ship Golden Bear*. The ship generally spends eight-months docked in Vallejo, California, taking occasional short trips in the Bay Area. The ship also takes two cruises for a total of four-months each year, often to remote locations such as the Far East or Australia. Being located on an ocean going vessel allows the Facility to perform shipboard testing while underway and land-based testing while secured in Carquinez Strait. The ship is owned by the U.S. Maritime Administration and is operated by Cal Maritime as part of the California State University (CSU) system. The GBF science team is from Moss Landing Marine Laboratory (MLML), also a part of the CSU system.

GBF was developed to conduct research, development, testing, and evaluation (RDTE) of technologies and operational practices that show promise for limiting the impact of marine vessel operations on the environment. Specifically, GBF provides:

- A dedicated onboard laboratory to enable rapid biological and chemical analysis to support RDTE activities.
- Access to all ship equipment; including the ballast water system, hull and apertures, bilge water and de-oiling equipment, sanitation system, and diesel engine exhaust systems.
- Specialized equipment to support ballast water treatment system evaluation, including a dedicated pump and piping system, a means of installing and integrating a treatment system, an automation system, and a water sampling system.
- Equipment for use in Land-based testing water augmentation, including biological concentrators, methods of various chemical delivery, and tank mixing systems.

Addition details are provided in the Facility Physical Plant Description (Reference 3).



Photo 1. *Training Ship Golden Bear* —Vallejo, California

Section 3 Ballast Water Treatment System (BWTS)

The Facility will receive a BWTS according to the specifications listed below. Variations to these specifications may result in project delays and a price increase to the client. The particulars described herein shall be provided by the client with the BWTS.

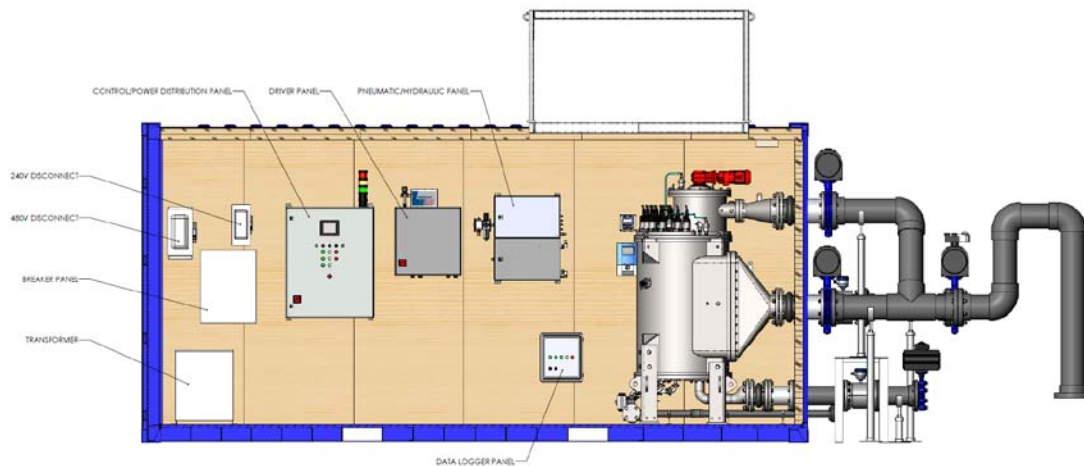


Figure 1. Ballast Water Treatment System

3.1 Technology Description

The BWTS has been designed to treat marine vessel ballast water through a combination of filtration and UV irradiation, up to 250 m³/hr. The BWTS utilizes both the filtration and disinfection steps as ballast water is taken up (ballast) into the marine vessel. The marine vessel will then hold the treated ballast water in its tanks for an undetermined period of time (hours to months). The ballast water is also sent to the BWTS when it is discharged (deballast) from the marine vessel ballast water tanks overboard to the sea. During deballast, the BWTS utilizes the UV irradiation disinfection, but bypasses the filtration system.

The BWTS consists of the following principal components:

Treatment Unit

- Stainless steel construction 316L containing:
 - 12 filter elements
 - 24 Low pressure high efficiency UV lamps (500 Watts each)
 - Automatic Filter Backwash system, removing material from the filtration screens and sending overboard through a waste connection
 - Automatic Lamp Sleeve cleaning system
 - UV Sensor

Pneumatic and Hydraulic Control Cabinet

- Contains all solenoid valves, pneumatic controls and hydraulic motor

Lamp Driver Cabinet

- Houses the drivers for the UV lamps and power disconnect switch.

Control Cabinet

- Houses the PLC and other system control function components. The control switches and HMI are located on the control door.
- Houses the main system power, fuses and breakers.
- Distributes power to the Lamp Driver cabinet(s), and Control Cabinet.

Automatic Filter Backwash System

The BWTS is equipped with an automatic filter backwashing system that automatically initiates a cleaning sequence based on differential pressure across the filtration system. The system has two pressure sensors, one located on the ballast water inlet and the other on the ballast water outlet. These pressure sensors provide a signal to the control panel to initiate a backwash sequence. The backwash sequence consists of opening an actuated backwash valve and signaling the filter drive motor to make one revolution. Each revolution of the drive motor allows each filter element to reverse its flow allowing accumulated debris trapped by the filter to be carried out to drain. As each individual filter element is being backwashed, the remaining filter elements continue to process water. Once the filter drive motor has completed its revolution, the backwash valve closes completing the cleaning sequence.

Automatic Lamp Cleaning System

The BWTS is equipped with a lamp cleaning system that automatically initiates a cleaning sequence at the beginning and end of a ballasting cycle. The lamp cleaning system removes any fouling that could build up on the lamp sleeves. The lamp cleaning sequence can also be initiated automatically or during operation.

The automatic lamp cleaning system is sequentially driven. Each lamp wiping mechanism consists of a wiper plate, wiping seals and one hydraulic actuator. The hydraulic actuator moves the wiper plate with wiping seals from one end of the lamp sleeve to the other. A cleaning sequence consists of actuating the lamp cleaning system from its home position to the end of the lamp sleeves and back to the home position. The cleaning cycle is finished when the last lamp wiping mechanism reaches its home position.

UV Sensor

The BWTS is equipped with a UV sensor to monitor the UV output of the lamps. In the event of low UV output, an alarm condition is initiated to warn of the potential for insufficient treatment.

Control System

The control system monitors key parameters allowing automatic: engagement of back flushing system, operation of BWTS valves, warning alarms, fault shutdowns, and logging of operating data. Backflushing is automatically logged and can be downloaded for Facility purposes.

During testing, BWTS equipment will be surveyed and documented for final reporting.

General Process Description

Ballasting

Water is pumped into the Ballast Inlet (Ballasting) at the top of the treatment unit. The water flows first through the filter elements, where debris and larger organisms are removed. An automatic on-line back flushing cycle removes debris trapped on the filter elements, and directs it back to the original source water through the backwash outlet.

Water passes through the filter elements and then flows through the UV treatment zone within the unit. The treated water is then directed to the ballast water pipe through the Ballast Water Outlet.

De-ballasting

Upon de-ballasting, water is sent directly to the UV treatment zone, and bypasses the filtration portion of the unit. Ballast water enters through the Ballast Water Inlet (de-ballasting) on the front of the unit. Water is directed through the UV treatment zone for a second dose of UV treatment, and then directed back to the ballast water pipe for discharge through the Ballast Water Outlet.

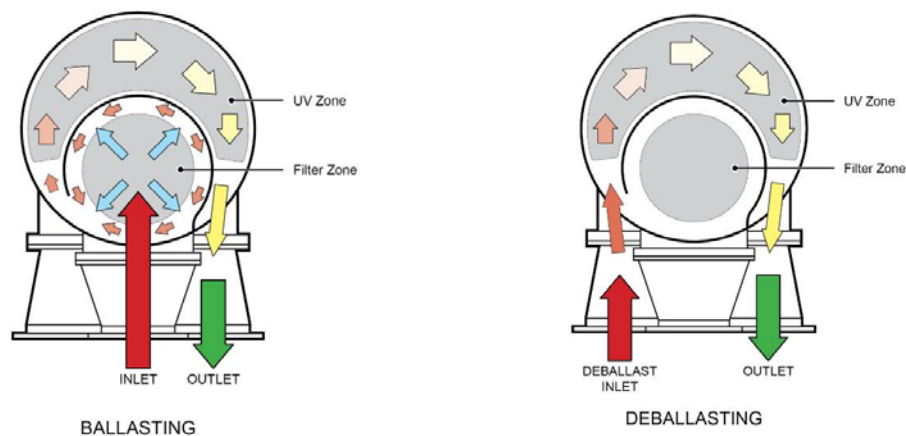


Figure 2. Ballasting and De-Ballasting

Table 2 - BWTS Particulars

Flow rate	0 – 250 m3/hr (+/- 10%)
Pressure	≥1.5 barg (Required at BWTS inlet) 2-6 barg working pressure
Filtration	30 µm
UV treatment	500 watt/lamp, 254 nm Lamp type: TrojanUV Solo Lamp™ Sensors: 1UV, 1 level, 1 temperature, 2 pressure
Interfaces	Structure, piping, electrical, and controls to meet Facility requirements.

3.2 Scope of Supply

A complete BWTS shall be provided as a factory-assembled package ready for integration with the Facility. The BWTS shall be suitable for operation by Facility staff. The BWTS shall be compatible with the below interface points:

- A complete assembly shall be supplied as a skid mounted unit. All equipment, including controls and auxiliary systems, shall be mounted on a 20-foot ISO rack or in a 20-foot ISO container. Alignment, dimensions, and position of the corner castings shall meet ISO R-668 or ISO R-1161.
- The assembly shall not exceed 25 short tons.
- One inlet and one outlet ballast water connection shall be provided. Each shall be 8-inch, 150-pound ANSI flanges, preferably flat faced and located on one end of the container.
- One flushing connection shall be provided. It shall be 4-inch, 150-pound ANSI flanges, preferably flat faced on the same end of the container as inlet and outlet.
- The BWTS electrical panel shall be set-up to interface with one or more of GBF's available electrical supplies. All power is 60 Hertz, alternating current, with a Russellstoll Cable Connector. GBF provides one (1) connection at 100 amps and 440 volts, 50 amps at 440 volts, and 30 amps at 120 volts. The BWTS shall include all required transformers and distribution of required power.
- National pipe thread connection to receive compressed air. Compressed air will be provided at 80 psig, and will not be conditioned. The BWTS shall include all required driers and pressure reducing stations if needed.

The BWTS shall be brought to a GBF-designated location on the Cal Maritime campus in Vallejo, California during standard working hours. At least 48-hours notice shall be provided to the Director prior to delivery.

Supply shall also include one (1) manufacturer's representative (engineer/technician) for five (5) days of on-site commissioning and training. The representative shall assume the responsibility for GBF personnel training during commissioning trials within the available time.

The client shall provide adequate on-site personnel and resources to make any repairs during commissioning, stress testing, and testing operations. All repairs will be witnessed by Facility staff and documented in the test report.

Following completion of testing, the director will notify the client that the BWTS will be removed from GBF and placed in a weather location on the Cal Maritime campus for collection.

3.3 BWTS Monitoring and Documentation

The BWTS shall be supplied with key documentation to support the installation, operation, and reporting of test results. In general this documentation should follow Section 5 – *Typical Documentation Requirements for the Plan Approval Process* and Part 1 – *Specifications for Pre-Test Evaluation of System Documentation* of the G8 Guidelines.

To meet the purposes of the Facility, the following documentation is required:

- Technical manual with installation check list, commissioning check list, operational limitations/specifications, and operating instructions.
- Drawings with particulars for system connection.
- List of major components including maker, model number, and key operating parameters. For example filter elements, UV lamps, monitoring devices.
- List of key operating parameters and limits that control system operation. This might include transmittance, flow rate, pressure, temperature, salinity.

The BWTS shall supply an electronic copy of all monitored parameters during testing. These parameters shall be equivalent to those identified in Section 4.10 through 4.14 – *Control and monitoring equipment* of the G8 Guidelines. This data shall be accessible to the Facility through memory stick download or other means. At the Facility discretion, this data will be downloaded on a weekly basis. During each test cycle, the BWTS and monitoring operations will be surveyed and documented.

Section 4 Organization

In order to achieve this BWTS project objectives, a coordinated team has been established with specific roles and responsibilities. This team is outlined in the organization chart shown below, and the responsibilities are detailed in the Facility's Quality Management Plan.

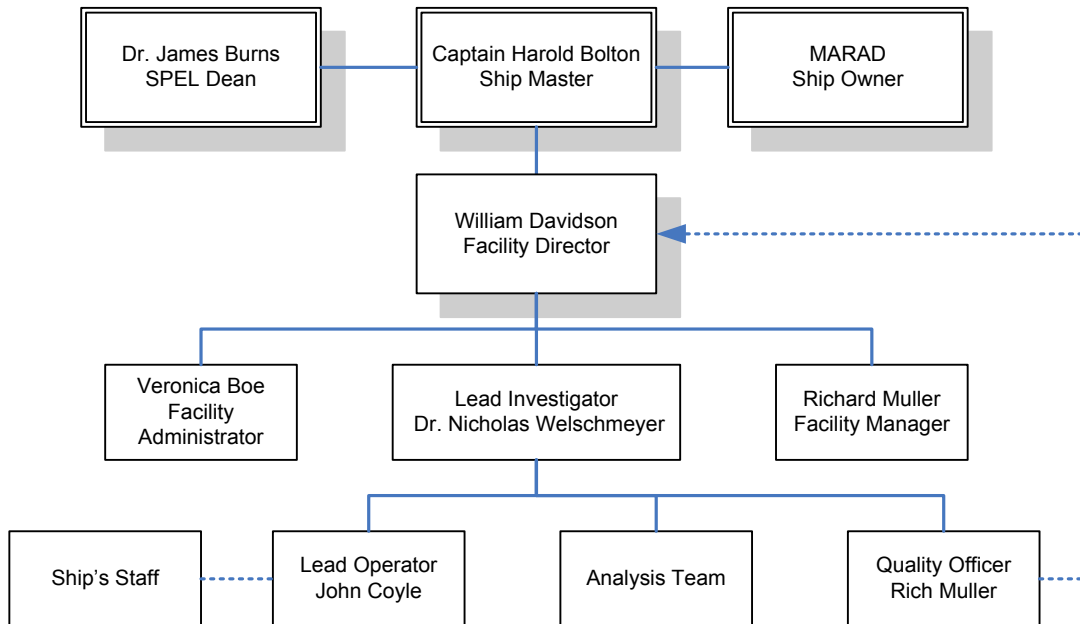


Figure 3. Facility Organization Chart

4.1 Project Roles and Responsibilities

Nicholas Welschmeyer, as Lead Investigator, will supervise the scientific staff. William (Bill) Davidson, Lead Operator, will supervise the ship's staff and will coordinate efforts in all phases of ballasting operations/sampling operations. For the most part, the scientific staff will have a 'hands-off' role in the mechanical operation of ballast pumps, valves, and flow strategy. Ship crew will identify the source water (e.g., uptake, control tank, or treatment tank) and provide flow, to be collected in appropriate vessels by the scientific staff.

In addition to the standard staff identified in the Quality Management Plan (QMP, see program documentation), the specifics of the Analysis Team are listed here. The Lead Investigator will oversee and participate in all phases of the scientific operation. The Lead Investigator will, specifically:

- Act as the prime scientific technician during the important sample collection events during each ballasting operation.
- Assign sample processing tasks to the remainder of the scientific crew and will be chief guardian of raw data (notebooks, data sheets, computer files, hard drive backups).
- Coordinate with the Quality Officer (Richard Muller of CMA), who will oversee data archiving and data distributions.
- Write the final Facility report.

Quality will review all Facility reports for conformance with the Data Verification Records. Discrepancies will be documented and provided to the Director.

Analysis for this testing includes the following personnel from MLML:

- Erin Jensen (BS Biology, MS Marine Sciences)
- Jules Kuo (BS Biology, MLML Masters candidate)
- Brian Maurer (BS Biology, MLML Masters candidate)
- Jeffrey Johnsen (BS Biology, MLML Masters candidate)

All microscope operations will be handled by Jensen and Kuo, both of whom are familiar with planktonic taxa. They will share workloads in bacterial pathogen determination and culture plate analyses. Maurer will handle assays involving flow cytometry, fluorescence determinations, and dilution-based MPN analysis of the 10-50 μm size class (Welschmeyer will assist).

Short term assistance for colony-counting and monitoring of MPN grow-outs at MLML will be provided by Johnsen. Assistance will also include vehicle transport, back-up technician duties, and project IT support.

See Appendix B for key personnel contact information.

Section 5 Project Objective and Description

5.1 Project Objective

The primary objective of the project is to complete a valid test of the BWTS employing IMO guidelines for Shipboard, and Land-Based testing. This objective is met through a battery of assays that quantitatively evaluate the number of living organisms in treated and untreated ballast water, where organisms are distinguished by size ($<10\ \mu\text{m}$, ≥ 10 to $<50\ \mu\text{m}$, $\geq 50\ \mu\text{m}$) and by organism type (e.g., specific pathogens).

5.2 Project Description

This section provides a brief description of the project. As per the following Plan Schedule, the project is divided into three phases: Documentation, Biological Efficacy Testing, and Reporting. The following sections provide an overview of each of these tasks and sub-tasks.

Subsequent sections discuss personnel and roles, effective protocols and methods, and challenge water conditions. Various facility documents are referenced throughout in an effort to avoid duplication.

5.3 Nomenclature

The facility uses specific nomenclature to describe various activities. The following provides definitions, and applies this nomenclature to Project Charlie.

Project. A Project is a collection of tests to verify a technology claim, or collection of experiments to prove a thesis. Project Charlie consists of four Shipboard tests and ten Land-Based tests to verify that the BWTS meets IMO D-2 standards. The Shipboard tests will be conducted with ambient water at various locations. The Land-based tests will consist of six brackish and six marine tests. The project will be performed in accordance to the IMO G8 Guidelines and the ETV protocol.

Test. A Test is one replicate activity that compares the performance of a treatment system (or other method) to a control. Each Project Charlie Test consists of a sequential treatment uptake through the treatment system and a control uptake with no treatment. These two separate ballast water parcels have similar challenge conditions as they are taken from the same ambient water source (Shipboard) or the same “source tanks” (Land-Based). The two parcels are held separately in a “treatment” tank and a “control” tank. The treatment is discharged through the treatment system after a designated hold time. The control is discharged without treatment after a similar hold time.

Event and Cycle. The Facility uses the term Event to describe a discrete combination of activities, typically performed without stopping, resetting, or other breaks. It typically takes several Events to complete a Test. For Land-based testing, the Facility uses the term Cycle to describe a series of events that accomplishes two tests.

5.4 Testing Sequence

Preparation

Preparation includes the following Events:

- BWTS Commissioning
- BWTS Stress Testing
- Tank Inspections

Shipboard Tests

Four (4) total Tests completed, at separate locations with different ambient water conditions.

Test 1. Uptake Oakland, CA. Discharge Vallejo, CA. Test 1 includes the following events:

- Initial Tank Cleaning
- Pipe Cleaning
- Treatment Uptake
- Control Uptake
- Pipe Cleaning
- Treatment Discharge
- Control Discharge

Test 2. Uptake Sacramento, CA. Discharge at sea. Test 2 includes the following events:

- Pipe Cleaning
- Treatment Uptake
- Control Uptake
- Pipe Cleaning
- Treatment Discharge
- Control Discharge

Test 3. Uptake Los Angeles, CA. Discharge at sea. Test 3 includes the following events:

- Pipe Cleaning
- Treatment Uptake
- Control Uptake
- Pipe Cleaning
- Treatment Discharge
- Control Discharge

Test 4. Uptake Vallejo, CA. Discharge Oakland, CA. Test 4 includes the following events:

- Pipe Cleaning
- Treatment Uptake
- Control Uptake
- Pipe Cleaning
- Treatment Discharge
- Control Discharge

Land-Based Brackish Water Tests

Six (6) total Tests completed in three Cycles.

Cycle A. Consists of Tests 1 and 2. Cycle A includes the following events:

- Tank and Pipe Cleaning
- Test 1 Treatment Uptake and Combined Test 1 and 2 Control Uptake (Note: a single control parcel serves two treatment Tests)
- Pipe Cleaning
- Test 2 Treatment Uptake
- Pipe Cleaning
- Test 1 Treatment Discharge
- Test 2 Treatment Discharge
- Combined Test 1 and 2 Control Discharge

Cycle B. Consists of Tests 3 and 4. Cycle B includes the following Events:

- Tank and Pipe Cleaning
- Test 3 Treatment Uptake and Combined Test 3 and 4 Control Uptake
- Pipe Cleaning
- Test 4 Treatment Uptake
- Pipe Cleaning
- Test 3 Treatment Discharge
- Test 4 Treatment Discharge
- Combined Test 3 and 4 Control Discharge

Cycle C. Consists of Tests 5 and 6. Cycle C includes the following Events:

- Tank and Pipe Cleaning
- Test 5 Treatment Uptake and Combined Test 5 and 6 Control Uptake
- Pipe Cleaning
- Test 6 Treatment Uptake
- Pipe Cleaning
- Test 5 Treatment Discharge
- Test 6 Treatment Discharge
- Combined Test 5 and 6 Control Discharge

Land-Based Marine Water Tests

Six (6) total Tests completed in three Cycles.

Cycle D. Consists of Tests 7 and 8. Cycle D includes the following Events:

- Tank and Pipe Cleaning
- Test 7 Treatment Uptake and Combined Test 7 and 8 Control Uptake
- Pipe Cleaning
- Test 8 Treatment Uptake
- Pipe Cleaning
- Test 7 Treatment Discharge

- Test 8 Treatment Discharge
- Combined Test 7 and 8 Control Discharge

Cycle E. Consists of Tests 9 and 10. Cycle E includes the following Events:

- Tank and Pipe Cleaning
- Test Nine Treatment Uptake and Combined Test 9 and 10 Control Uptake
- Pipe Cleaning
- Test 10 Treatment
- Pipe Cleaning
- Test 9 Treatment Discharge
- Test 10 Treatment Discharge
- Combined Test 9 and 10 Control Discharge

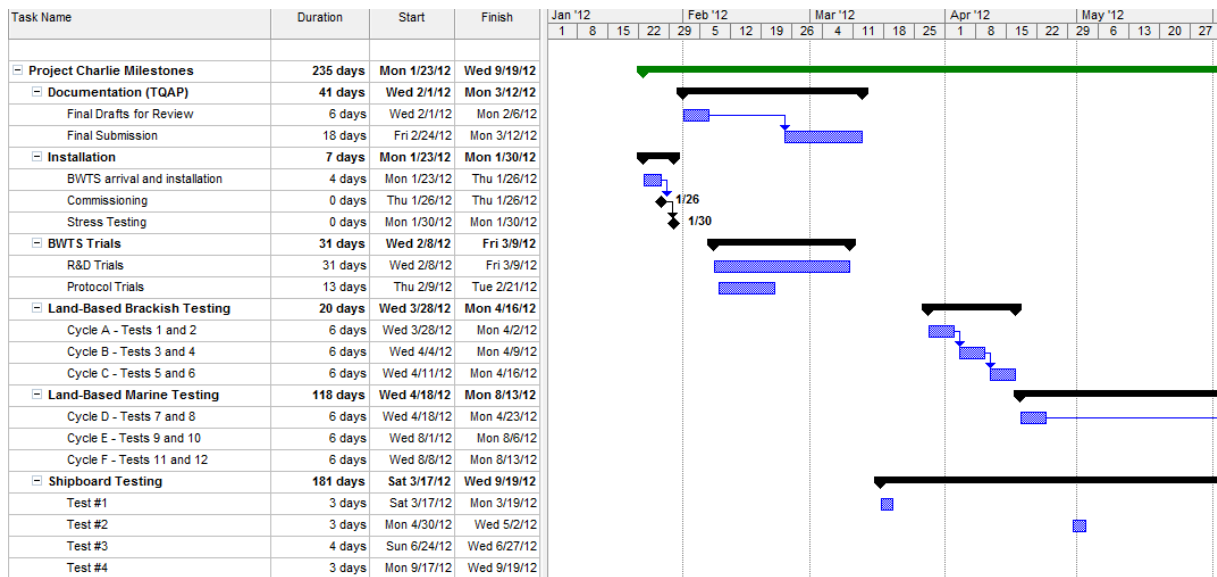
Cycle F. Consists of Tests 11 and 12. Cycle E includes the following Events:

- Tank and Pipe Cleaning
- Test 11 Treatment Uptake and Combined Test 11 and 12 Control Uptake
- Pipe Cleaning
- Test 12 Treatment Uptake
- Pipe Cleaning
- Test 11 Treatment Discharge
- Test 12 Treatment Discharge
- Combined Test 11 and 12 Control Discharge

5.5 Project Schedule

Project Charlie Shipboard testing has been schedule for dates in March, April, June, and September. Project Charlie Land-Based testing has been scheduled for March through September. The two segments of testing will overlap. The Facility is ready to receive the BWTS, and installation and commissioning can be performed in one (1) calendar week. Shipboard trials must span a period of not less than six (6) months, and will occur prior to, and after Land-Based testing. To gain a total of twelve Land-Based tests, only ten being required, requires six (6) calendar weeks. Decommissioning and removal, after all testing is complete, takes less than one week. Total required project period spans 32 weeks Land-Based testing is split between two periods of four (4) consecutive calendar weeks. The below Project Schedule accounts for holidays, onsite client R&D trialing, and provides contingency periods.

Table 3 – Project Schedule



A Land-Based test cycle consists of two (2) tests including uptake, treatment, and discharge. The Facility pairs two treatment tests with a single control. Each test cycle takes one (1) calendar week, typically from Wednesday to Monday, allowing the weekend to include the in tank hold period. In general the weekly schedule will consist of:

- Friday – Facility and team orientation, preparation, and documentation.
- Monday – Ballast water uptake to Source Tanks, biology and water quality augmentation.
- Tuesday – Continued Source Tank augmentation and final sampling.
- Wednesday – Source Tank uptake to Treatment 1, Control, Treatment 2, sampling and start of analysis.
- Thursday – Test water holding and continued analysis. Hold time will be ~120 hours.
- Monday – Test water discharge (Treatment 1, Treatment 2, and then Control), sampling and start of analysis. Ballast water uptake to Source Tanks.

Daily schedules will be developed as needed by the Facility to support daily operations. These schedules will be provided to the project team as they are developed. During a testing operation, a red-line mark-up of the daily schedule is expected and will become a log of the day's events. In general the daily routine will follow the above general pattern.

5.6 Documentation

With a focus on quality control and quality assurance, the following documents are provided to the Verification Organization (VO). GBF's documentation control process is detailed in the standard operating procedures of the Quality Management Plan (QMP).

The Test Quality Assurance Plan (TQAP) contains the project-specific documentation submitted for VO review, comment, and eventual approval, which includes the:

- **Project Plan.** This is a project-specific plan that identifies the testing objectives and conditions, describes the testing facility and equipment to be evaluated, and establishes the testing logistics, including personnel and schedule.
- **Land-Based and Shipboard Quality Assurance Procedures Plans (QAPP).** Each QAPP contains quality assurance/quality control measures established for executing the SOPs to test the treatment system, including operating parameters and data collection requirements. In addition, the protocols for evaluating test biological and chemical conditions are established.
- **Standard Operating Procedures (SOPs).** These procedures are for operations and sampling that are specific to this testing.

GBF Program Documentation submitted for VO reference only includes the:

- **Environmental, Health, & Safety Plan (ES&H).** The ES&H includes GBF programmatic standard operating procedures (SOPs) that govern operations for the protection of the environment, and the health and safety of GBF and adjacent personnel.
- **Quality Management Plan (QMP).** The QMP provides GBF standard operating procedures (SOPs) for managing data, including collection techniques, error corrections, verification, and storage. The QMP also describes GBF's plant and sampling system.
- **Facility Physical Plant Description (FPPD).** The FPPD provides a detailed description of the physical aspects of the GBF, including information about the ballast water pumping system, ballast water treatment system services station, onboard marine laboratory, and the automation plant.

The project's final report will also be submitted to the VO for review, comment, and acceptance.

5.7 Verification Organization Inspection/Audit

The Verification Organization (VO) is expected to perform at least one on-site inspection of the testing. During this testing, the VO will document that the BWTS unit being tested is in conformance with the BWTS supplied documentation including major equipment components and operating parameters. The VO is also expected to perform an audit of the Facility procedures and reports.

5.8 Biological Efficacy Testing

The biological efficacy (BE) testing consists of receiving, installing, and commissioning the BWTS, running a complete set of brackish condition events and marine condition events, Shipboard testing events, and finally removing the system from the GBF site.

The QAPPs provide a detailed description of required inspections and commissioning procedures for the BWTS. This includes a shakedown test of the BWTS, which has the purpose of stressing the system so that any failures are apparent and can be remedied prior to the BE testing.

In the case that the BE testing is not successful due to facility malfunction, a contingency period of two weeks is allotted for make-up testing efforts.

Section 6 Experimental Approach

Testing has been designed to meet the intent of the guidelines specified by International Maritime Organization (IMO). The experimental approach does not consider operations and maintenance, reliability, cost factors, environmental acceptability, or safety factors.



Photo 2. Facility Laboratory Work

6.1 Quality Control and Quality Assurance

All operations must occur within the requirements of the Facility Quality Management Plan and both QAPPs. This compliance will include at a minimum:

- Utilization of the facility's automation and online record system to the maximum extent possible. At a minimum, all valve positions, flow rates, and sampling rates will be recorded, logged, and stored at one-minute intervals.
- Duties of the Quality Officer will include verification of all collected data sheets, and scanning of hand logs into the online record system on a daily basis.
- Use of standard operating procedures for pumping system use and sample collection techniques.

6.2 Automation System and Online Information System

The features of GBF's automation and online information system are outlined in the Facility Physical Plant Description and the QAPP. The automation system serves to minimize the errors that occur from manual user entry, provide visual cues to GBF personnel to facilitate activity timing and system operational modes, and provide secure information storage and access.

The automation system includes the following features:

- Passive automatic monitoring and logging of most system parameters, such as valve positions, ballasting flow rates, pressures, temperatures, water chemistry characteristics, and sampling flow rates.
- Active control of certain system devices, such as BWTS start and stop, GBF pumping rate, and key valve operators.
- Automatic logging of BWTS electrical load, voltage, and frequency on 1-minute basis.
- User interface screens providing pictorial representation of system current operation; visual cues to GBF staff to help determine time until the tank will be full and pumping rates.
- Information access to procedures and protocols at any of the user terminals located throughout GBF.
- Data entry forms, for on-line entry of various records and forms.
- Automatic generation of predesigned data reports, based on collected data. For example, system flow rate over time.

6.3 Challenge Water

Shipboard

Biological Efficacy (BE) testing will occur in March, April, June, and September. Uptakes will occur at various locations in California inland and coastal Waters, during the *T.S. Golden Bear's* 2012 summer training cruise. All testing will be performed with ambient water, without adding or concentrating of any chemical or biological components.

The challenge water living populations must exceed ten times (10x) the concentration of organisms in treated discharge as permitted in IMO D-2 standards. There are no specific water quality requirements for Shipboard testing.

Land-Based

Brackish and Marine (salt) condition BE testing will occur in March-April and September 2012 with water taken up from the Carquinez Straits at a depth of approximately seven (7) meters. The challenge water quality requirements and the living population requirements are listed in the tables below. Carquinez Strait water quality does not typically meet these minimum requirements, therefore, two GBF tanks have been transformed into "Source Tanks." The water quality of these Source Tanks can be augmented to meet various challenge water requirements. Parameters that can be augmented are:

- Salinity
- Dissolved Organic Carbon (DOC)
- Particulate Organic Carbon (POC)
- Total Suspended Solids (TSS)
- Living Populations

Given the use of ambient water prior to Source Tank augmentation, the following steps are conducted to time ballast uptake to maximize potential to meet challenge water criteria. The figures, December Zooplankton Tidal Cycle Counts and December Phytoplankton Counts, below are provided for guidance.

1. The science team will identify the general tidal period for ballast water uptake based on previously collected data.
2. The system will be placed in Source Uptake mode, according to the procedures.
5. Source Tanks will be sampled immediately upon filling, via Niskin bottle. Analysis will be conducted within 24 hours to quantify challenge water characteristics and living populations.

Table 4. Water Quality Requirements – Land-Based Brackish Source Water

Salinity	10 – 20 PSU
Temperature	4 – 35 Celcius
DOC	6 mg/L as DOC minimum
POC	5 mg/L as POC minimum
TSS	50 mg/L minimum

Table 5. Water Quality Requirements – Land-Based Marine Source Water

Salinity	>32 PSU
Temperature	4 – 35 Celcius
DOC	6 mg/L as DOC minimum
POC	4 mg/L as POC minimum
TSS	25 mg/L minimum

Table 6. Living Populations—Land-Based Source Water

Organism Size Class	Total Concentration	Diversity
$\geq 50\mu m$	10^5 organisms/m ³	5 species across 3 phyla
$\geq 10\mu m < 50\mu m$	10^3 organisms/mL	5 species across 3 phyla
$< 10\mu m$	10^4 /mL as culturable aerobic heterotrophic bacteria	5 species across 3 phyla

Table 7. Living Populations—Land-Based Control Discharge

Organism Size Class	Minimum Concentration
$\geq 50\mu m$	100 organisms/m ³
$\geq 10\mu m < 50\mu m$	100 organisms/mL
$< 10\mu m$	5×10^2 /mL as culturable aerobic heterotrophic bacteria

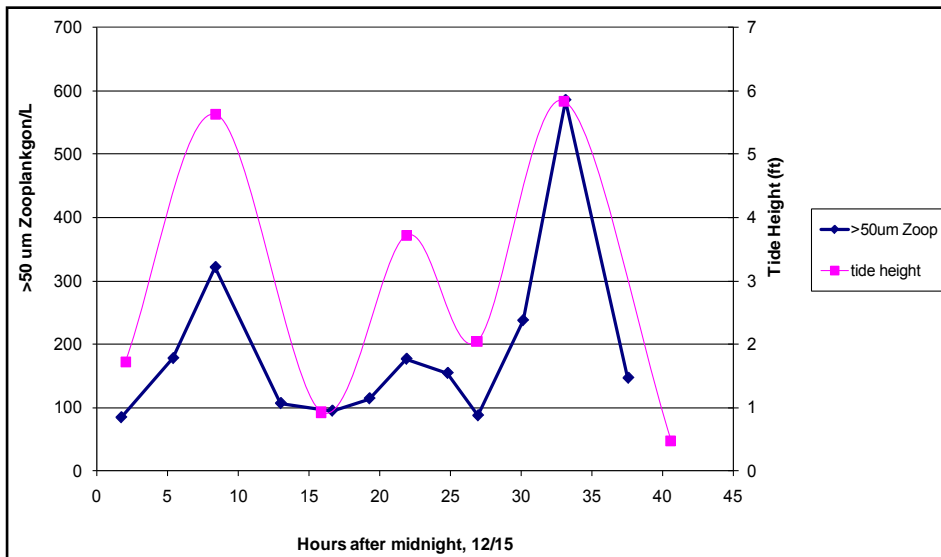


Figure 4 – December Tidal Cycle Live Zooplankton

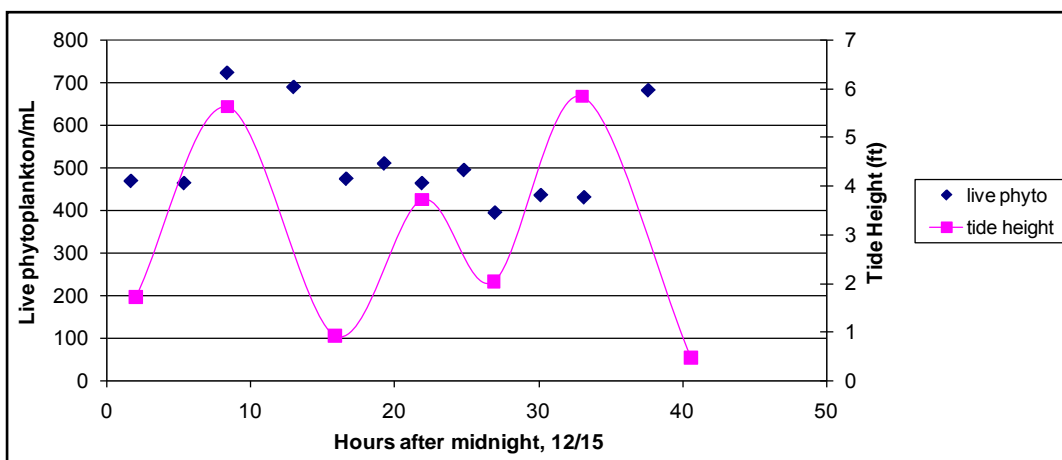


Figure 5 – December Tidal Cycle Live Phytoplankton

6.4 System Commissioning and Tank Flushing

The BWTS will be installed and commissioned in accordance with the manufacturer's requirements. In addition, the system will undergo a shakedown test.

In order to ensure that the BE testing cycles are valid, the facility piping system and the treatment and control tanks will be cleaned and flushed with a hypochlorite solution, and then drained in accordance with the procedures in the QAPPs.

6.5 Biological Efficacy Testing and Valid Test Parameters

The QAPPs and SOPs provide detailed overview and instructions on the biological efficacy testing. The test validation parameters for Shipboard and Land-Based testing are listed in the tables below. These parameters include: engineering values such as flow rates and total

volumes; water quality values such a salinity and temperature; and biological criteria such as zooplankton counts. The parameters are measured during Shipboard and Land-Based testing, at each of the steps in a test or cycle as follows:

Shipboard

- Treatment/Control Uptake. Ensure ambient water meets biological requirements, in addition to flow rates, volumes, and sampling volumes.
- Treatment Tank Discharge. Must meet maximum biological requirements, flow rates, flow rates, and sample volumes.
- Control Tank Discharge. Must meet minimum biological requirements, flow rates, and sample volumes.

Land-Based

- Treatment Tank 1 uptake. Ensure Source Tank water meets challenge conditions, in addition to flow rates, volumes, and sampling volumes.
- Control Tank uptake. Ensure Source Tank water meets challenge conditions, in addition to flow rates, volumes, and sampling volumes.
- Treatment Tank 2 uptake. Ensure Source Tank water meets challenge conditions, in addition to flow rates, volumes, and sampling volumes.
- Treatment Tank 1 discharge. There are no water quality requirements. Must meet maximum biological requirements, flow rates, and sampling volumes.
- Treatment Tank 2 discharge. There are no water quality requirements. Must meet maximum biological requirements, flow rates, and sampling volumes.
- Control Tank discharge. There are no water quality requirements. Must meet minimum biological requirements, flow rates, and sample volumes.

Table 8 – Valid Test Parameters, One Shipboard Test

	Criteria	Uptake Treatment	Cycles Control	Discharge Treatment	Cycles Control
Treatment Line and Tank					
Average (m3/hr)	250 ± 10%				
Total Volume (m ³)	≥ 200				
Control Line and Tank					
Average (m3/hr)	250 ± 10%				
Total Volume (m ³)	≥ 200				
Combined Sample Volume (m³)					
Uptake and Control Discharge	≥ 3				
Treatment Discharge	≥ 9				
Ballast Hold Duration (hours)	48+				
Water Quality					
Salinity (PSU)	N/A				
Temperature (Celcius)	N/A				
DOC (mg/L)	N/A				
POC (mg/L)	N/A				
TSS (mg/L)	N/A				
Uptake Living Populations					
≥50 microns (organisms/m ³)	100				
≥10 < 50 microns (organisms/mL)	100				
Control Living Populations					
≥50 microns (organisms/m ³)	10				
≥10 < 50 microns (organisms/mL)	10				

Table 9 – Valid Test Parameters, One Land-Based Cycle

	Criteria		Uptake Events			Discharge Events		
			Treat 1	Treat 2	Control	Treat 1	Treat 2	Control
Treatment Line and Tanks								
Average (m³/hr)	250 ± 10%							
Volume at end Cycle (m³)	200 (-0%/+10%)							
Control Line and Tank								
Total Volume (m³)	200 (-0%/+10%)							
Combined Sample Volume (m³)								
Uptake	≥ 1							
Control Discharge	≥ 3							
Treatment Discharge	≥ 9							
Ballast Hold Duration (hours)	120 (-0%/+10%)							
Water Quality	Brackish	Salt						
Salinity (PSU)	10 - 20	>32						
Temperature (Celcius)	4 - 35	4 - 35						
DOC (mg/L)	≥ 6	≥ 6						
POC (mg/L)	≥ 5	≥ 4						
TSS (mg/L)	≥ 50	≥ 24						
Uptake Living Populations								
≥50 microns (organisms/m³)	10^5							
≥10 < 50 microns (organisms/mL)	10^3							
<10 microns (bacteria/mL)	10^4							
Control Living Populations								
≥50 microns (organisms/m³)	100							
≥10 < 50 microns (organisms/mL)	100							
<10 microns (bacteria/mL)	500							

Sample Volumes (Note 1)

The sampling system includes three (3) tubs that provide triplicate integrated samples over the entire period of the uptake and discharge periods. The minimum sampling criteria is based on each individual sample volume.

The sampling period is intended to cover the entire ballast water uptake and ballast water discharge. However, there will be a gap or an overlap in sampling during starting of sampling, and during time when nets are being switched should one become too full.

Before uptake, the pumping system is placed into sea-to-sea such that sampling can be set-up and be ready to start. It then takes careful coordination to start sampling at the same time that the pumping team starts to actually fill a tank. The following review shows that there is no contamination risk in starting “early” or “late” only a skewing of representativeness.

- Early sample on uptake. The recirculated challenge water in the piping gets sampled until the ballast tanks actually start getting filled. This challenge water will be going into the tanks, so they are valid to sample.

- Late sample on uptake. The sampling misses some of the challenge water that is being taken up. It is expected that the challenge water conditions will not significantly change during a brief gap between start of uptake and start of sampling.
- Early on tank discharge. The water that will be recirculated is from the bottom of the ballast tank to be discharged. The sample representativeness will be skewed toward the bottom contents of the tank.
- Late on tank discharge. The sampling misses some of the tank ballast water that is being discharged. A brief gap is not expected to significantly alter the results of the organism counts.

Sample Rates (Note 2)

The sampling rates are based on a relationship between the main ballast water velocity, and the size of the pitot tube that takes the sample. This is to gain a representative sample from the ballast water main. According to protocol, the pitot tube design typically varies the diameter between 1.5 and 2.0 times the isokinetic diameter. Translated into volumetric flow rates, this would be roughly +/- 30% variation in flow rate. The sampling team will be using flow meters to control the flow of the samples on an instantaneous basis within this range.

Test Duration

Test duration for Shipboard testing will be not-less-than 48 hours. This is on a start-to-start basis, from the start of Treatment uptake, to the start of Treatment discharge. Test duration for Land-Based testing will be at least 120 hours + 10%. This is on a start-to-start basis, from the start of the Treatment 1 uptake event, to the start of the Treatment 1 discharge event. The discharge of the Control Tank time is not a validation parameter, but its discharge will generally start within three (3) hours of the completion of discharging the treatment tanks. An example schedule for Land-Based testing follows:

- Wednesday Uptake of Source Tanks to Treatment 1.
 Uptake of Source Tanks to Control.
 Uptake of Source Tanks to Treatment 2.
 (Estimated 3-5 hours total duration)
- Monday Discharge of Treatment 1.
 Discharge of Treatment 2.
 Discharge of Control.
 (Estimated 3-5 hours total duration)

6.6 Test Validation Review (Failures)

Once a test event is started in most cases it should be completed. For example, if the target salinity is in range at the start of uptake, but part way through the event it is no longer within target parameters, the team will continue to take up the ballast water until the event is complete. For example, if a tank discharge has started and a sampling net breaks, the team will work to recover as best as possible while the uptake is completed.

Following each event, uptake or discharge, the team will work to validate that the test has been completed accurately. If a parameter has been missed, the team will contact the

Verification Organization for consultation. The consultation will determine if the test cycle is still valid, or is not based on if the test will still accomplish the project objective:

Complete a valid test of the BWTS employing VO guidelines.

In the case that the variation or stoppage of a test event is due to equipment failure, in particular the treatment system, the test will be immediately stopped. Reasonable efforts will be made to repair the faulty equipment following manufacturer's guidance. Following reasonable remedy, the system may be run for some time in recirculation mode to prove the soundness of the repair. The test may then continue as planned.

In the case that the stoppage impacts maximum hold times for biological sampling, these samples may be processed in two batches – one before the failure, and one after the failure.

6.7 Decommissioning and Reporting

Unit will be removed following the completion of all Shipboard and Land-Based testing.

Draft and final reports will be issued in accordance with the Project Schedule in 5.5.

Section 7 Environmental, Health & Safety Plan

All operations must occur within the requirements of the GBF Environmental, Health & Safety Plan (EH&S Plan). This compliance will include, at a minimum:

- Updating of the Vessel General Permit to reflect the BWTS installation.
- Training of all GBF personnel, particularly the Science Team, in facility safety procedures.
- Compliance with all confined space entry procedures and documentation when entering, inspecting, and cleaning tanks.
- Compliance with the requirements of the U.S. Coast Guard and American Bureau of Shipping for all machinery installation and operation, particularly electrical connections and ground protection.

7.1 GBF EH&S Plan

The guidelines apply to GBF personnel or vendor bringing hazardous materials onboard the *Training Ship Golden Bear*. This guideline does not supersede the Cal Maritime EH&S Policy.

7.2 References

- University -National Oceanographic Laboratory System (UNOLS), Research Vessel Safety Standards, March 2009.
- Moss Landing Marine Laboratory (MLML) Emergency Response Plan.

7.3 Hazardous Materials

A hazardous material is any substance or combination of substances that, because of quantity, concentration, physical, chemical, radiological, explosive, or infectious characteristics, poses a substantial present or potential danger to humans or the environment. Generally, such materials are classified as:

- Flammable liquids and solids.
- Oxidizing materials.
- Corrosive materials.
- Flammable and non-flammable compressed gases.
- Poisons or toxic substances.
- Disease-causing agents.
- Combustible liquids.
- Other Regulated Materials (ORM) (Department of Transportation (DOT) Hazard.
- Class “ORM”), including hazardous wastes.

Hazardous materials will be found among both ship and scientific stores and include such items as organic solvents, corrosives, compressed gases, flammable liquids, and toxic or reactive chemicals. Material Safety Data Sheets (MSDS) contain a list of product ingredients, indicating information about the type of hazard; recommended personnel protection and precautions; spill or leak procedures; and fire, explosion, health (including first aid), and

reactivity data; and most importantly, an emergency telephone number for assistance in the event of an accident. Employers are required to inform employees of what hazardous materials are present in the work place and train them, with the aid of the MSDS, in their proper use and handling (per 29 CFR 1910).

The Lead Scientist or the equipment vendor will be responsible for providing the following information to the Quality Control Officer prior to testing at/on the GBF:

- A list of all hazardous materials by chemical name, common name, UN identification number, type and classification of hazard, quantity (size of containers and number of each size container), user name and contact information.
- MSDS sheets for all materials listed above.
- A list of the spill response materials and the amount to be brought aboard to address spills or accidents.

7.4 Lithium Batteries

Lithium batteries require special fire extinguishing capabilities, depending on the type of material used in the manufacturing process.

7.5 Storage Containers

Material should remain in their original shipping containers (as received from the vendor) with labeling intact.

7.6 Compressed Gases

Compressed gases must be securely held to the ship structure with metal brackets or positive cargo straps. Ropes or other similar lashings must be avoided. All gas cylinders must have their safety cap in place, unless they are in use with a regulator.

7.7 Spill Response

Kits or materials to address spills or accidents are supplied by the user/vendor. The amount of material brought aboard must be sufficient to address a spill of the entire amount of the specific materials being brought aboard. A chemical spill kit should be located in the marine biological laboratory, as well as near any specific equipment attached to GBF where practicable.

7.8 Sharps Disposal

Syringes, sharps, and hypodermic needles brought on board should be treated as a safety hazard and proper provisions should be made for their safe use and disposal.

7.9 Responsibility

Basic responsibility for safety should follow the attached EH&S Policy for the California Maritime Academy. There may be time during a voyage or while dockside, however, when a quick response is needed.

7.10 Contacts—EH&S Response

During an emergency EH&S response, the Captain and Chief Engineer are to be notified immediately. Contact information is located in Appendix B.

Appendix A: California Maritime Academy EH&S Policy



POLICY NO. 206.3

ISSUE DATE: 1 April 1997 REVISION DATE: August 21, 2001	POLICY: Environmental Health & Safety
REFERENCE:	
APPROVED: /s/ William B. Eisenhardt	

Environmental Health and Safety Policy

It is the policy of the California Maritime Academy that a high level of safety consciousness be instilled in all campus activities, shipboard practices, and off campus events on a continuing basis through appropriate training and continuous emphasis and supervision. This safety consciousness applies to visitors, students, staff, and faculty.

The safety philosophy of the California Maritime Academy is:

Safety is everybody's responsibility

All injuries are preventable

Safe work practices must be the first consideration when performing any task

Every employee and student must be aware of their responsibility to address unsafe work behaviors and conditions

Training of personnel is an essential element of injury prevention

Visitors and contractors are required to comply with Academy safety policies

Responsibilities

The President oversees the effectiveness of campus health and safety practices. Through the campus supervisory personnel, safety policies are implemented and enforced in all facilities and operations.

Every member of the campus community including students, faculty and staff has the authority and responsibility to request that an operation perceived to be unsafe be shut down.

To assist the President and senior management in implementing and monitoring campus safety policy, an Environmental Health and Safety Committee (EH&S) is established and responsible for:

Keeping themselves current on laws and regulations governing safety, health and environmental protection and insuring that the campus complies with applicable regulatory requirements

Developing and implementing procedures for holding managers, supervisors and employees accountable for environmental, health, and safety requirements

Verifying by frequent inspection of campus equipment and facilities, that safety is a high priority in conducting the business of the institution.

Committee Membership

The composition of the committee will be as follows:

Representatives from:
Academic Affairs
Marine Programs
Health Center
Human Resources
Employee Unions
Students

The committee will be chaired by the Special Consultant to the President on an interim basis.

Training

Managers are responsible for insuring that appropriate training is conducted for all personnel subject to the following exposures:

General Safety Awareness
Head Injury (hard hat protection)
Hearing Injury (ear protection)
Sight Injury (eye protection)
Back Injury (lifting support)
Respiratory Injury (breathing protection)
CPR
Electrical Shock (tag out procedures)
Falls/Ladder Safety (emergency first aid)
Burns (emergency first aid)
Hazardous Spills (contamination prevention/emergency first aid)

Appendix B: Contact Information for Key Golden Bear Facility Personnel

Contact Information Golden Bear Facility Personnel

Last Revised 2 December 2011

Emergency Contacts (listed in notification order)

1. **William T. Davidson, Facility Director**
Golden Bear Facility
707.654.1304, cell 707.592.4267
bdavidson@csum.edu
2. **Harry Bolton, Ship Master**
California Maritime Academy
707.654.1192, cell 707.246.7495
HBolton@csum.edu
3. **Cal Maritime Emergency Operations Center**
707.654.1111
4. **EH&S Contacts—Moss Landing Marine Laboratories (MLML)**
831.771.4400
5. **Jocelyn Douglas, MLML Health and Safety Officer**
831.771.4451, cell 831.750.9563

Additional Project Contacts

Richard S. Muller, Quality Officer
Golden Bear Facility
707.654.1258
rmuller@csum.edu

Veronica Boe, Facilities Administrator
California Maritime Academy
707.654.1156
vboe@csum.edu

Nicholas A. Welschmeyer, PhD, Lead Scientist
Golden Bear Facility
831.771.4439
welschmeyer@mlml.calstate.edu

Jad Mouawad, Verification Officer
Det Norske Veritas
+47 480 50 902
Jad.mouawad@dnv.com

Test Quality Assurance Plan – Project Charlie
Volume II: Quality Assurance Project Plan (Land-Based
Testing)

Golden Bear Facility
Vallejo, California

Dept. 76408, Fund 46408
9 April 2012, Rev. A



Contents

Section 1	Summary	1
Section 2	Project Roles and Contact Information	2
Section 3	Data and Information	3
3.1	Facility Nomenclature Breakdown.....	3
3.2	Data Control	3
	Roles and Responsibilities.....	3
	Automation System and Online Information System.....	3
	BWTS Data Collection.....	4
	Documentation Verification	4
	Test Cycle Automation Initiation, Tracking, and Error Logging	4
	Red Lining of Procedures.....	5
	Hand Log Tracking and Filing	5
	Online Log Tracking and Filing	6
	Verification Data Record.....	6
	Report Data Verification.....	6
3.3	Data Quality Indicators.....	7
	Engineering Data	7
	Water Quality	9
	Biological Analysis.....	10
3.4	Data Reporting and Data Reduction.....	12
Section 4	Facility Preparation	13
4.1	Challenge Water Verification.....	13
	Continuous Data	13
	Grab Samples.....	13
4.2	Tank Cleaning and Inspection.....	13
	Tank Cleaning.....	13
	Final Inspection	14
4.3	Source Tank Preparation	14
	Source Tank Filling	14
	Source Tank Mixing	14
	Organism Concentration $\geq 50 \mu\text{m}$ Size Class.....	15
	Organism Augmentation $\geq 10 \mu\text{m}$ $< 50 \mu\text{m}$	15
	Water Quality Augmentation.....	15

Final Sampling.....	15
Section 5 BWTS Commissioning	16
5.1 Arrival Inspection.....	16
5.2 Installation.....	16
5.3 Service.....	17
5.4 Operational Check.....	17
5.5 BWTS Shakedown Test	17
5.6 Lay-Up.....	18
Section 6 Biological Efficacy Test Procedures	19
6.1 Test Day Roles	19
6.2 Test Validation Parameters.....	20
6.3 Treatment System Operation.....	21
Standby	21
Ballasting Operation.....	22
Back Flushing Cycle.....	22
De-Ballasting Operations.....	22
6.4 Pumping and Piping Operation	22
Flushing and Draining Prior to Testing	22
Treatment Tank 1 Uptake	23
Treatment Tank 2 Uptake	24
Control Tank Uptake	24
Flushing and Draining Between Fill and Discharge.....	24
Treatment Tank 1 Recirculation and Discharge	24
Treatment Tank 2 Recirculation and Discharge	25
Control Tank Recirculation and Discharge	25
6.5 Sampling Procedures.....	25
Sampling Overview	26
Sampling Coordination.....	27
6.6 Water Quality Analysis	28
Continuous Data	28
In-Situ Probe Measurements.....	28
Grab Samples.....	29
6.7 Biological Efficacy Analysis.....	31
Organisms Larger than 50 µm	31
Organisms Larger than 10 µm, but Smaller than 50 µm – Required Assays	32

Organisms Larger than 10 µm, but Smaller than 50 µm – Corroborative Assays.....	33
Organisms Less than 10 µm	34
Section 7 Water Toxicity Testing	36
Overview	36
Sample Collection.....	36
Toxicity Testing.....	36
Reporting	36
Section 8 Assessments	37
 Appendix A: Project Training Requirements	
Appendix B: Toxicity Testing Conditions and Criteria	
Appendix C: Summary of Toxicity Test Conditions	

References

- 001 *Environmental, Health, & Safety Plan*, Golden Bear Facility, October 2010.
- 002 *Quality Management Plan*, Golden Bear Facility, December 2011.
- 003 *Physical Plant Description*, Golden Bear Facility, October 2010.
- 004 *Project Plan for Project Charlie*, Golden Bear Facility, March 2012.
- 005 *Quality Assurance Project Plan (Shipboard Testing) – Project Charlie*, Golden Bear Facility, March 2012.
- 006 *Standard Operating Procedures – Project Charlie*, Golden Bear Facility, March 2012.
- 007 *Protocol for the Verification of Ballast Water Treatment Technologies*, US Environmental Protection Agency, September 2010.
- 008 *Guidelines for Approval of Ballast Water Management Systems (G8)*, Annex 4, MEPC 174(58), Marine Environment Protection Committee, 10 October 2008.

Section 1 Summary

The Quality Assurance Project Plan (QAPP) establishes the quality assurance/quality control measures for executing the SOPs, the operating parameters and data collection requirements, and the protocols for evaluating test biological and chemical conditions. This QAPP is specific to the evaluation of the Project Charlie ballast water treatment system (BWTS) for Land-Based testing in accordance with the International Maritime Organization (IMO) *Guidelines for Approval of Ballast Water Management Systems (G8)* and the US EPA Environmental Technology Verification (ETV) protocol to the extent reasonable.

The QAPP is one part of the Test Quality Assurance Plan (TQAP) that also consists of Project Charlie specific Project Plan (Plan) and Standard Operating Procedures (SOPs). This QAPP also references the Facility Quality Management Plan (QMP). Wherever a conflict exists between documents, the SOPs takes first precedence and the QAPP takes second precedence.



Photo 1. Facility Operations

Section 2 Project Roles and Contact Information

Project roles and responsibilities are as described in the Program Documentation *Quality Management Plan*. The key personnel contact information for this BWTS project is as follows:

William T. Davidson, Facility Director/Test Coordinator

Golden Bear Facility
707.654.1304, cell 707.592.4267
bdavidson@csum.edu

Richard S. Muller, Facility Manager/Quality Officer

Golden Bear Facility
707.654.1258
rmuller@csum.edu

Veronica Boe, Facilities Program Administrator

California Maritime Academy
707.654.1156
vboe@csum.edu

Nicholas A. Welschmeyer, PhD, Lead Investigator

Golden Bear Facility
831.771.4439
welschmeyer@mlml.calstate.edu

Harry Bolton, Ship Master

California Maritime Academy
707.654.1192, cell 707.246.7495
HBolton@csum.edu

Jad Mouawad, Verification Organization

Det Norske Veritas
+47 480 50 902
Jad.mouawad@dnv.com

Andrew Daley, BWTS Project Manager

Trojan Technologies
519.457.3400, cell 226.688.7430
adaley@trojanuv.com

ES&H Contacts—Moss Landing Marine Laboratories (MLML), 831.771.4400

Jocelyn Douglas, MLML Health and Safety Officer, 831.771.4451, cell 831.750.9563

Section 3 Data and Information

This section outlines the procedures involved in the control of, quality indicators for, and reduction validation of project data and information.

3.1 Facility Nomenclature Breakdown

The facility uses specific nomenclature to describe various activities. The following provides definitions, and applies this nomenclature to Project Charlie.

Project. A Project is a collection of tests to verify a technology claim, or collection of experiments to prove a thesis. Project Charlie consists of five brackish and five salt water tests to verify that the BWTS meets the IMO D-2 standard. The project will be performed in accordance to the IMO G8 Guidelines and where possible the guidance of the ETV protocol.

Test. A Test is one replicate activity that compares the performance of a treatment system (or other method) to a control. Each Project Charlie Test consists of a sequential treatment uptake through the treatment system and a control uptake with no treatment. These two separate ballast water parcels have similar challenge conditions as they are taken from the same “source tanks.” The two parcels are held separately in a “treatment” tank and a “control” tank. The treatment is discharged through the treatment system after a designated hold time. The control is discharged without treatment after a similar hold time.

Event and Cycle. The Facility uses the term Event to describe a discrete combination of activities, typically performed without stopping, resetting, or other breaks. It typically takes several Events to complete a Test. The Facility uses the term Cycle to describe a series of events that accomplishes two tests.

3.2 Data Control

Each project related task is tracked either through the automation system, online information system, or hand logs which are later scanned into the online information system.

Roles and Responsibilities

The Quality Officer (Quality) reports directly to the Facility Director. Quality is responsible for the data and information procedures outlined in this section, and detailed in the various Standard Operating Procedures (SOPs). The Lead Operator (Operator) is responsible for all pumping and piping operations. The Lead Scientist (Scientist) is responsible for all sampling and analysis operations. The Test Coordinator (Coordinator) provides logistics and timing assistance to the team. The Operator and the Scientist, while they do not report to Quality, are responsible to provide Quality with the data and information outlined in this section. Facility personnel roles are further explained in the Facility Environmental, Safety & Health Plan, and the Facility Quality Management Plan.

Automation System and Online Information System

The automation system is outlined in the Facility Physical Plant Description. The automation system is comprised of a computer network which monitors and securely records a wide array of field sensors such as valve position indicators, sample flow meters, and water quality

instruments. User interfaces are located through-out the Facility to provide visual indicators piping system, BWTS, and sampling system status to the Facility team. Further, the automation system facilitates the generation of reports based on the stored data.

The online information system is a computer network for handling Facility documentation. Access is enabled for personnel to view standard and test specific procedures, access secure collected data, and manipulate data into useful information for reports. User forms allow online entry of data directly into the information system. Hand logs are scanned into the system.

BWTS Data Collection

The BWTS is expected to have its own data collection system in accordance with the IMO G8 Guidelines. This data shall be downloaded and transferred to the Facility information system not less than once per week.

Documentation Verification

In consultation with the Director, Science, and Operations, Quality will confirm the correct versions of the Facility standard documents, and project specific documents. Quality will track this in the datasheet, and confirm the correct versions are available online.

Quality, prior to the start of a test cycle, will determine which aspects of the automation system will be used for data monitoring, and where the Facility team will need to maintain hand logs. To make this assessment, Quality will open, view, practice, and determine the availability of each of the data collecting tools in the automation system.

Upon this determination, Quality will update the Quality Data Sheet to select which data collection will be by Hand Log and which will be Online.

Quality will distribute the documentation list to all team members.

Test Cycle Automation Initiation, Tracking, and Error Logging

A test cycle is one complete ballasting evolution including: uptake to two treatment tanks and one control tank, a holding period, and finally discharge of the treated and control ballast water.

Before start of a test cycle, the Operator and Scientist will provide an automation and online system status report to Quality. The report will include: calibration report for field instrumentation; system check of all monitored parameters; system check of all screens, forms, and reports. An action plan for hand monitoring and logging of non-functional items will be submitted. Quality will scan the report into the online information system.

At the start of a test cycle, before any data logging or pumping activities, Quality will initiate the automation to begin data logging. The automation system will automatically generate a unique test event number and begin logging of all monitored parameters. This unique number will be matched to all monitored data and all online data sheets in the online system. The automatically generated test event number will be based on the date and time of the start of the effort. For example, if initiation occurs at 1:32 pm on March 14th, 2012, the test number would be: "12-03-14-1332."

Before the test event, Quality will perform the automation system checks as identified in the data log. In addition, Quality will communicate with the Operator and Scientist to identify any automation system errors. Automation errors found independently, or highlighted by team members, will be documented and made part of the data record. Where possible, hand records of that monitored parameter will replace the automated entry.

At the conclusion of the test event, the Quality Officer will gain confirmation from the Lead Operator and stop the automatic collection of data.

Red Lining of Procedures

As testing efforts progress, the complexity of the operations and variability of the equipment and challenge water conditions, it may at times be advantageous or imperative to change procedures.

For example, the treatment system following a back flush cycle fails to open its valve. In this case, the Operator may use ordinary means, such as manually opening the valve, to allow the test to continue.

For example, a sampling net fails allowing some part of its contents to spill into the sample tub. In this case, the Scientist might continue sampling to that net, but analyze its contents separately from the other two nets, and not average results until it is determined that all results are consistent.

In any case, where there is a deviation from the test procedures, protocol, plan, etc. the person-in-charge of that operation must “red-line” that document. Redlining is accomplished by:

- Strike a single line, using indelible ink, through the affected text, figure, chart, or other item.
- Initial each adjacent to each and every strike.
- Provide, using indelible ink, any required correction in the form of text, figure, chart, or other item.
- Provide an explanation in the “notes” section of why the change was made.
- Date and sign each affected page.

Hand Log Tracking and Filing

At the end of each test cycle, the Quality Officer will manually collect all hand logs from team members, and scan these into the online information system. Each hand log will be assigned the test number and an identifier associated with that log. For example, the BE sampling log for the above test event would be: “2012-03-14-1332-BESAMPLING.”

Daily during the test cycle, the Quality Officer will view automated reports and online data logs to confirm that the systems are working correctly.

The Quality Officer will then continue to collect additional hand logs as they are completed, scanning these into the online information system.

Online Log Tracking and Filing

Daily during the test cycle, the Quality Officer will review and track all online logs using the Quality Data Sheet. A copy of utilized logs will be copied to a secure location in the information system, and named using the same convention as for the hand logs.

Verification Data Record

Following each test cycle, the Quality Officer will make one (1) copy of the automation system database, the scans of hand data, and the online data forms to a secure read only DVD. At the end of a Test Cycle two (2) copies will be made. Each DVD will be scribed with test cycle name, date, and copy recipient. For example, a DVD for test day March 16th, would read: "Test Cycle 12-03-14-1332, Data Recorded 12-03-16, Operator Copy." The DVD copy will be immediately distributed to: the Facility Director for off-site storage and the second copy kept on-site by Quality.

Report Data Verification

Quality will review all Facility reports for conformance with the Data Verification Records. Discrepancies will be documented and provided to the Director.

3.3 Data Quality Indicators

Statistical analyses will be carried out on data obtained for all performance measurements. As part of the assessment of data quality, six data quality indicators (DQIs) will be used to interpret the degree of acceptability or utility of the data. Data quality will be reported in a table comparing these objectives and criteria, to recorded results. The following protocol will be used to assess the following DQIs, as well as acceptable limits and criteria for:

- Representativeness
- Accuracy
- Precision
- Bias
- Comparability
- Completeness

At the conclusion of each test, the data will be processed and compared to the below assigned DQI targets. These are not Pass/Fail targets, but rather an indicator of the quality of the collected data. Where data does not meet its target, an explanation is required. In subsequent tests, either the procedure will be modified to improve data quality, or the target will be lowered.

Engineering Data

Engineering data is collected and stored by the automation system for post processing. In general, the data follows expected trends through each test event.

For example, recorded tank levels will follow the trend of the recorded pumping rate. A rate of 250 cubic meters per hour should result in a level corresponding to 250 cubic meters of ballast water in the tank after one hour.

For example, recorded temperatures should agree with the water quality monitoring system and there should be general agreement between the various sensors in the system. If the temperature monitor that is recording at the pump discharge reads 20 Celsius, and the temperature monitor at the treatment system discharge reads 10 Celsius this does not follow the expected trend, and needs to be investigated.

The instrumentation is calibrated by a combination of factory set points, and practical tests. For example, the sample tubs have graduated marks against which the sample flow meters total volume counters can be compared.

Table 1 - Data Quality Indicators for Engineering Parameters

Parameter	Indicator	Metric	Description
Ballast Water Flow - Rosemount 8711 Flow Meter	Representativeness	90%	Flow meters are continuously online and recording. A temporary system fault is possible.
	Accuracy	+/-3%	Instrument performance is +/-0.3% of flow as per factory calibration.
	Precision	0.1	Cubic meters per hour. Display on unit.
	Bias	NA	
	Comparability	10%	All ballast water passes at least two of the three flow meters, allowing a comparison between them.
	Completeness	90%	Data is automatically recorded in the automation system.
Ballast Water Volume - TMS LevelCom Tank Level Indicator	Representativeness	90%	Indicator measures the water column in the forward portion of the tank. If ship is trimmed aft, it will miss a small portion of this water when the tank level is very low.
	Accuracy	+/-5%	Some variation is expected due to the shape of the tanks changing at various heights.
	Precision	0.1	Meters. This is a function of the system analog signal to the automation system.
	Bias	NA	
	Comparability	10%	Variation when compared to flow meter and time calculation while tank is being filled/emptied.
	Completeness	90%	Data is automatically recorded in the automation system.
Sample Water Flow - Seametrics SPX	Representativeness	90%	Flow meters are continuously online and recording. A temporary system fault is possible.
	Accuracy	+/-5%	Instrument performance is +/-1% of full scale.
	Precision	0.3	Gallons per minute on display unit.
	Bias	NA	
	Comparability	10%	Variation between the three sample tubs that take simultaneous samples.
	Completeness	90%	Data is automatically recorded in the automation system.

Water Quality

Water quality measurements will be conducted with a combination of field installed continuous monitoring equipment, and discrete sampling conducted by either hand held in-situ instrumentation or laboratory analysis.

The continuous monitoring provides real time viewing and verification, as well as a continuously recorded set of readings.

Table 2a – Continuous Monitoring Parameters

Sensor	Maker	Type	Calibration Procedure	Reporting Units	Range	Accuracy	Precision
Temperature	Sea-Bird Electronics	SBE 21	Nominally 1/year	degrees C	-5 to +35	0.01	0.001
Conductivity	Sea-Bird Electronics	SBE 21	Nominally 1/year	S/m	0 to 7	0.001	0.0001
Transmissometer (25 cm)	Wet Labs	C-Star	Nominally 1/year	% transmittance or beam attenuation	0-100	N/A	N/A
Dissolved Oxygen	Sea-Bird Electronics	SBE 43	Nominally 2/year	mg/L	120% of saturation	N/A	N/A
Fluorometer	Turner Designs	Cyclops-7	In-house, before each experimental run	ug/L	0.1 to 50	+/- 5%*	0.01

* When compared to wet chemistry.

Table 2b – Grab Sample Monitoring Parameters

Parameter	Maker	Type	Calibration Procedure	Reporting Units	Range	Accuracy	Precision
Temperature	YSI	6560	Nominally 1/year	Degrees C	-5 to +50	0.15	0.1
Conductivity	YSI	6560	Nominally 1/year	S/m	0 to 100	0.5%	0.1
Dissolved Oxygen	YSI	6150	Nominally 1/year	mg/L	0 to 50	0 to 20: ± 0.1 or 1%, whichever is greater	0.01
Chlorophyll α	YSI	6025	In-house per exper. run	$\mu\text{g/L}$	0 to 400	0.1 $\mu\text{g/L}$ Chl	~ 0.1
Turbidity	YSI	6136	In-house per exper. Run	NTU	0 to 1000	± 0.1	0.1 NTU
pH	Beckman	70	In-house per exper. Run	pH units	0 to 14	± 0.1	± 0.03
Total Suspended Solids (TSS)	Proweight	Filter	Calibrated 1/year, checked per exper. run	mg/L	5 to 100	± 2	2.60%
Particulate Organic Carbon (POC)	GF/F	Filter	In-house per exper. Run	mg/L	0.1 to 100	± 0.15	7.50%
Dissolved Organic Carbon (DOC)	GF/F	Filter	Per exper. run, at McCampbell Analytical, Inc.	mg/L	0.5 to 100	± 0.1	20%

Biological Analysis

Data quality indicators (DQIs) are determined empirically by the GBF science staff based on actual sample measurements made in the GBF laboratory. By design, the Facility has developed procedures that generate a high level of replication.

Table 3 - Data Quality Indicators for Engineering Parameters

Sample Description	Analytical method	Data Quality Indicators (DQI)*	Notes
1. Organisms >50 µm (#/m ³)	Visual determination of live status (30x magnification)	Expected CV** <31%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
2a. Organisms 10–50 µm Method A MPN (MPN/mL)	MPN determination of cultivable phytoplankton; fluorometric determination of growth	Expected CV <60%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
2b. Organisms 10–50 µm Method B Flow cytometry (#/mL)	Flow cytometric determination of live cells tagged w/ fluorescein diacetate (FDA)	Expected CV <42%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
2c. Organisms 10–50 µm Method C Epifluorescence microscopy (#/mL)	Visual epifluorescence determination of live cells tagged w/ FDC and CMFDA	Expected CV <25%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities (experience limited to one complete ballast sequence)
3a. Organisms < 10 µm Bulk bacterial plate counts (CFU/mL).	Total colony forming units (CFU) on agar substrate	Expected CV <90%.	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
3b. Pathogens <i>E. coli</i> (MPN/100 mL)	MPN determination IDEXX proprietary, enzyme-based MPN kit (Colilert™)	Expected CV <64%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
3c. Pathogens <i>Enterococci</i> (MPN/100 mL)	MPN determination IDEXX proprietary, enzyme-based MPN kit (Enterolert™)	Expected CV <42%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
3d. Pathogens <i>Vibrio cholerae</i> serotype 01 (detection limit <1CFU/mL)	Antibody detection; New Horizons Diagnostics, Cholera Smart™ II	N.A.	No observation of <i>V. cholera</i> (Type 01) >1 CFU/100 mL has been found in any sample analyzed at GBF (n=36)
3e. Pathogens <i>Vibrio cholerae</i> serotype 0139 (detection limit <1CFU/mL)	Antibody detection; New Horizons Diagnostics, Bengal Smart™ II	N.A.	No observation of <i>V. cholera</i> (Type 0139) >1 CFU/100 mL has been found in any sample analyzed at GBF (n=36)

* Data quality indicator (DQI) is estimated as twice the empirically measured coefficient of variation (CV) for sample replicates analyzed by GBF science staff for the given assay.

**CV = Standard Deviation/mean*100, where the sample standard deviation is

$$\sqrt{\sum(x - \bar{x})^2 / (n - 1)}$$

3.4 Data Reporting and Data Reduction

All test data for BWTS testing will be collected, analyzed, and reported in a uniform format. Specifically, all data from time-integrated sample collections; e.g., net samples and time-integrated microbe/chemistry ‘grab’ samples, will be collected in triplicate, corresponding to the three individual sampling ports configured at the biological sampling station. Additionally, each replicate sampling container, e.g., each net and each 20 L ‘grab’ carboy, will be sampled in triplicate, again, for sample aliquots corresponding to the assays described in the Science SOPs.

Sample means and sample standard deviations will be calculated and reported as figures and tables as shown in the example dataset below (the number of replicates will be indicated). Note that the example below represents data reporting formats for the same raw data, e.g., the figure is a graphical representation of the tabular data. In effect, each ballasting sequence (uptake, control, treatment) will yield 3x3=9 analyses of the stated scientific measuring parameters. Example formats for data reporting are provided below.

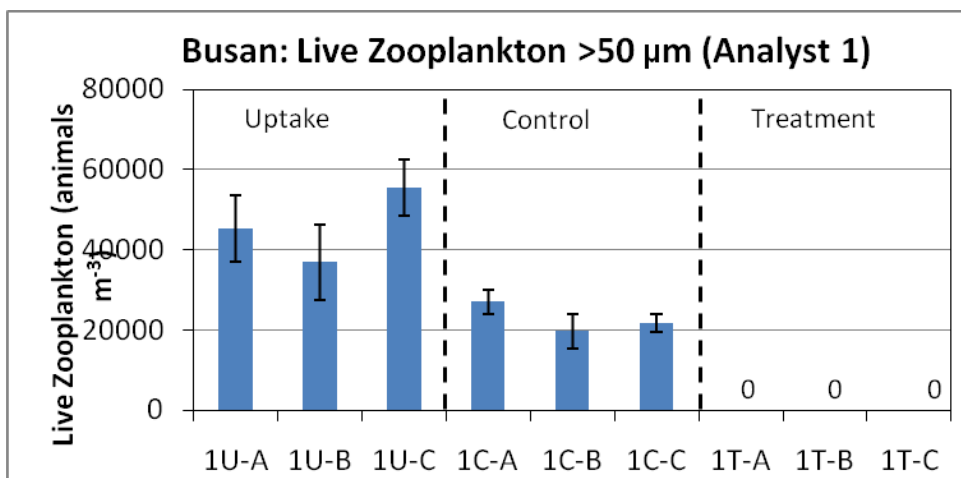


Figure 1 - Data Reduction Figure Example

Table 4 - Data Reduction Table Example

Location	Sample	Live Zoop. (#/m ³)		Dead Zoop. (#/m ³)		% Live	
		Mean	S. Dev.	Mean	S. Dev.	%	n
Busan (Uptake)	1U-A	45425	8282	23523	3351	65.7	3
	1U-B	36907	9328	17643	3219	67.4	3
	1U-C	55564	6965	18048	3337	75	3
Busan (Control)	1C-A	27004	3096	16956	5042	61.9	3
	1C-B	19684	4219	12845	2128	60.3	3
	1C-C	21750	2110	11136	1437	66	3
Busan (Treatment)	1T-A	0	0	4857	1052	0	3
	1T-B	0	0	3879	433	0	3
	1T-C	0	0	6649	823	0	3

Section 4 Facility Preparation

This section provides specific activities that the Facility will conduct in preparation for initial testing and efforts between testing cycles.

4.1 Challenge Water Verification

Continuous Data

Natural water for the BWTS will be collected directly from the Carquinez Straits (Northern San Francisco Bay) while *T/S Golden Bear* is at its California Maritime Academy (CMA) berth. This water will be stored in two “Source Tanks”. The water quality and living populations in these Source Tanks will then be augmented to meet both IMO and Environmental Technology Verification (ETV) Program requirements for brackish conditions and marine (salt) conditions, as described in the Project Plan. The criteria to be augmented are salinity, Dissolved Organic Carbon (DOC), Particulate Organic Carbon (POC), Total Suspended Solids (TSS), zooplankton, and phytoplankton. The Facility’s augmentation, mixing, and sampling processes are described in the next section.

GBF operates an inline Sea Bird SBE 21 thermosalinograph during land-based ballast testing. The data, including temperature, conductivity, salinity, dissolved oxygen, transmissivity (WetLabs Transmissometer) and chlorophyll fluorescence (Turner Cyclops), is displayed for the user in real-time on a computer screen and recorded to disk. The data is post processed according to Sea Bird’s recommendations.

Grab Samples

Grab samples of the challenge water during testing is described later in the *Biological Efficacy Testing* section of the QAPP.

4.2 Tank Cleaning and Inspection

Following guidance in the data sheets provided in the SOPs, the GBF Lead Operator and Quality Officer will ensure proper tank conditions are achieved prior to commencing testing and between testing cycles. Testing protocols require that the Source tanks (3-174-1, 3-174-2), Treatment tanks (3-154-1 and 3-154-2), and the Control tank (6-E-0) be opened, cleaned as required, inspected, and approved by the Lead Operator as “ready for testing” before each testing cycle commences. The GBF Lead Operator will contract with a tank cleaning contractor with all proper certifications and coverage for work aboard the vessel.

Tank Cleaning

The tanks will be opened at manhole access covers for each tank (2 each), the covers shall have safety rails installed, and mechanical portable ventilators shall be installed at each tank’s weather deck vent terminus. The tanks shall be ventilated continuously throughout the entire tank cleaning process. After no less than 12 hours of ventilation, a certified marine chemist will test the tanks as “safe for men” and tank cleaning can commence. The tanks will be checked by a certified competent person for continued safe condition at the beginning and end

of each work day. All tank entry documentation shall be maintained for inspection at the tank entry point.

An initial inspection by the Lead Operator and contractor after the first rinse will determine how much, if any, mucking and disposal of silt or debris is required. The silt will be mucked and loaded to appropriate containers staged on deck for later disposal as per local requirements.

Cleaners outfitted with appropriate Personal Protective Equipment (PPE) will use high pressure washers to spray down the treatment tanks surfaces with a 200 ppm chlorine solution. After a contact time of not less than five minutes, all surfaces will be rinsed with city fresh water using the same high pressure washers. The wash down from the cleaning will be continuously pumped out of the ballast tank and transferred to municipal waste.

A final inspection by the Lead Operator and contractor will confirm the removal of silt, debris, and wash water. In addition, random sampling of six (6) wet tank surface locations will be conducted. A reading of less than 3 ppm free chlorine is considered passing.

Final Inspection

The treatment tanks will be pumped “dry,” with no standing water, and left ventilating for an additional 24 hours. Final inspection will be performed by the GBF Lead Operator, Quality Officer, or assignee for acceptance, at which time the tank will be closed up and ventilation removed. The tanks will be offered for inspection to the BWTS representative and the Verification Organization prior to closing.

4.3 Source Tank Preparation

The Source Tanks (Tank 3-174-1 and Tank 3-174-2) will be filled, augmented with biological constituents, augmented with water quality constituents, and tested to meet brackish or marine condition criteria. All augmentation processes are conducted to achieve a +20% margin over the required concentration criteria. Guidance for preparing the Source Tanks is given in detail in the SOPs.

Source Tank Filling

The Source Tanks will be filled simultaneously with ambient water from the Carquinez Strait. Source Tank Uptake will be coordinated to occur during High Tide, thereby increasing the natural concentration of organisms present in the water. The water is sampled during uptake at the Facility’s inline sample ports, and tested for concentration of organisms in the $\geq 50 \mu\text{m}$ size class. The water is also tested for water quality constituents, as described below. If organism concentrations are adequate, the Source Tanks will be partially emptied to prepare for organism concentration.

Source Tank Mixing

The Source Tanks must be mixed during tank augmentation and technology testing to keep the water homogeneous and all non-dissolved particles suspended. A gentle air-mixing system will be operated in both tanks, providing continuous circulation. All materials and organisms added to the tanks will become homogeneously distributed throughout the tank volumes. The tanks will be sampled prior to uptake, and continuously sampled from the

uptake stream during testing. Three sequential samples are taken during uptake, and will be evaluated for homogeneity.

Organism Concentration $\geq 50 \mu\text{m}$ Size Class

A Facility concentrator system will be ran continuously after Source Tank uptake to augment the concentration of $\geq 50 \mu\text{m}$ organisms (zooplankton) in the Source Tanks. The concentrators will take water from the Facility's treatment piping system, at the sampling ports. The duration of this operation is dependent on the measured concentration of organisms during Source Tank filling. The Source Tanks are sampled via Niskin Bottle to determine if the desired concentration has been reached. The sample will also be tested for concentrations in the $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$ size class (phytoplankton) to guide a separate augmentation process.

Organism Augmentation $\geq 10 \mu\text{m}$ $< 50 \mu\text{m}$

Following the concentration of zooplankton, phytoplankton will be added to the Source Tanks. Phytoplankton is grown out from local waters in tubs located at the Facility. The tub contents are added to the tanks at the direction of the science team and the Source Tanks are sampled to assure the required concentration has been met. It is important to complete this step following the concentration of organisms $\geq 50 \mu\text{m}$, as the concentration process will accumulate phytoplankton as well as zooplankton.

Water Quality Augmentation

The water sampled during Source Tank Filling will be analyzed for constituents relating to water quality. The water is tested for salinity level, and concentrations of Dissolved Organic Carbon (DOC), Particulate Organic Carbon (POC), and Total Suspended Solids (TSS). The results are used to calculate the quantity of supplemental material for each constituent to be added. DOC is augmented with *Camellia sinesis*, which is found in NESTEA® "Unsweetered Decaf 100% Ice Tea Mix." POC and TSS are both augmented with the humic material Mesa Verde Resources "Micromate." TSS is expected to meet challenge conditions once POC minimums are achieved, but TSS may be raised with fine clay particulates contained in *Arizona Test Dust* as required.

After weighing out the calculated amounts of Ice Tea and Micromate to be added, the materials will be independently diluted with water taken off of the inline sampling ports during organism concentration. The POC and DOC/TSS-rich solutions will then be poured into the mixing Source Tanks. The tanks will continue to mix as concentration is ongoing.

Final Sampling

Upon completion of the augmentation processes, the tanks will be sampled a final time via Niskin Bottle to confirm that all criteria are met. In the event that one or more criteria does not meet IMO requirements, the corresponding augmentation procedure(s) will be repeated. If the two tanks' contents differ significantly, they will be homogenized via a designated pumping operation that will move water between the two tanks.

Section 5 BWTS Commissioning

This section provides specific procedures required for commissioning the BWTS. A successful commissioning will integrate the system with the Facility, and confirm proper mechanical operation in accordance with the BWTS instructions.

All commissioning efforts are performed by the Lead Operator (Operator). Checklists and other documentation are provided to the Quality Officer (Quality) on a daily basis. Commissioning data sheets are provided in the Standard Operating Procedures (SOPs).

5.1 Arrival Inspection

Upon arrival of the BWTS at the facility staging area, an initial inspection will be performed to determine delivery condition and readiness for install. Utilizing the “BWTS Commissioning Data Sheets,” the GBF Lead Operator or assignee, the rigging and installation contractor, and Quality will inspect the system for:

- Overall condition and damage indication.
- Condition of the ISO container rack.
- Ballast water inlet and outlet connections (8-inch flange).
- Drain connection (4-inch flange).
- Electrical load requirements.
- Cable lengths and sizes for GBF-provided connectors.
- Fresh water and compressed air requirements.
- Any additional requirements or conditions requiring specific care or address.

5.2 Installation

Utilizing the “BWTS Commissioning Data Sheets,” the GBF Lead Operator or assignee will ensure that the BWTS is properly installed, secured, and that all mechanical, electrical, and plumbing connections are made up.

Based on standard requirements for installing a 20-foot ISO container on the designated location onboard the *TS Golden Bear* and any unique requirements identified at the arrival inspection, the GBF Director will utilize a sub-contract to:

- Provide crane, rigging, and mechanical service to shift and secure the BWTS to the vessel
- Install all plumbing interfaces, including ballast in and out, drain, air, and water, along with any unique interfaces such as chemical injection or sampling lines.

Crane and rigging services will be planned and scheduled with facility and vessel personnel at least 24 hours prior to commencement, to allow for notification and alternate arrangements for planned events. A suitable mobile crane and operator will be employed, along with a rigging crew to shift the BWTS from the CMA waterfront parking lot to the end of the pier near the stern of the vessel. The interference radius of the vessel’s aft crane shall be marked

out, and suitable blocking placed to allow the mobile crane to lift and set the ISO container onto the 01 Aft Deck of the vessel just outside this turning radius. The contracted rigging crew and crane operator will then employ the vessel's crane to shift the container into position and correct alignment for placement and securing to the container platform. The vessel's Chief Mate will observe, inspect, and approve the securing of the container.

Electrical power interfaces will typically be installed by qualified facility personnel under direction of Chief Engineer, though an electrical contractor with facility oversight may be employed. Depending on the complexity of the BWTS user interface, the inter-connection to the Integrated Monitoring and Control system (IMACS) will be performed by qualified facility personnel or contracted to the vessel's automation contractor.

5.3 Service

Utilizing guidance from the BWTS Technical Bulletin and the "BWTS Commissioning Data Sheets," the GBF Lead Operator or assignee will ensure that the system will be inspected and serviced by facility personnel and/or an OEM Technical Representative under facility supervision prior to placing BWTS in operation. In general, this service would include inspection of UV lamps, filter installations, freedom of mechanical linkages and pump shafts, operation of fitted valves and handwheels, and control interface condition. Any installed lubrication points shall be checked or lubricated as per BWTS Technical Bulletin.

5.4 Operational Check

Utilizing guidance from BWTS Technical Bulletin and the "BWTS Commissioning Data Sheets," the GBF Director or assignee will initiate operation of the BWTS. The system will be placed into operation readiness by checking all system elements for proper installation and gradually bringing them on line, which will include the following checks:

- The booster pump will be checked for proper rotation.
- Piping connections and covers will be checked under system pressure.
- Flow rates will be determined and confirmed.
- Electrical power and controls integration will be initiated and confirmed.

5.5 BWTS Shakedown Test

Shakedown subjects the BWTS to stressing conditions that are within the BWTS's specified limits. The purpose is to ensure that the BWTS will not have mechanical failures during BE Testing. The shakedown steps are detailed in the "BWTS Shakedown Test Data Sheets" located in the SOPs, and are outlined below:

- Sea-to-Sea for two (2) hours at ~250 m³/hr treatment.
- Starts and stops of facility pump and BWTS.
- Overnight idle period.
- Sea-to-Sea for four (4) hours at ~275 m³/hr treatment (110% of capacity).
- Uptake cycle operational test to determine expected conditions
- Two (2) hour uptake sampling test to determine expected plankton net conditions

5.6 Lay-Up

After commissioning and shakedown test, and between test cycles, the system will be laid up as per the “BWTS Commissioning Data Sheets.” The GBF Director or assignee shall oversee the performance of these lay-up procedures based on Technical Bulletin recommendations and guidance. In general, these procedures will consist of flushing the system with fresh water and opening power supply breakers to the BWTS, thus leaving in “wet” lay-up while installed on the vessel. For idle periods in excess of 1 month or if extended freezing periods are expected, the lay-up will also consist of draining the unit and filter on the BWTS.

Section 6 Biological Efficacy Test Procedures

This section provides the procedures for the biological efficacy testing cycle. This includes ballast water uptake and discharge procedures, sampling collection and analysis procedures. The section is divided into the following parts:

- Test day roles.
- Test validation parameters.
- Treatment system operation.
- Pumping and piping operation.
- Sampling procedures.
- Water quality analysis.
- Biological efficacy analysis.

6.1 Test Day Roles

The conduct of a biological efficacy test requires careful coordination of pumping and piping systems, treatment system operations, sample collection, sample analysis, water quality continuous monitoring, water quality grab samples, and most importantly quality control. To facilitate these operations, the following identifies the roles of key personnel related to completing these tasks.

Given the small size of the Facility, persons will play multiple roles. As such, the below description is listed by person.

Bill Davidson.

- Test Conductor. Ensures that treatment system operation, pumping and piping operations, and sampling collection activities are coordinated and properly timed.
- Equipment Responsible Party. Responsible for all mechanical equipment involved with pumping and piping.
- Operator Supervisor. Reviews testing plan with Operator such that the pumps and piping system are properly operated.

Rich Muller.

- Quality Officer. Ensures that the procedures being utilized are current, and that all documentation is completed and logged into the automation system. Starts and stops the event. Checks function of the automation system during a test.
- Water Quality – Continuous Monitoring. Ensures that the continuous water quality system is calibrated, operating, and properly logging data. Provides feedback on water quality to the Scientist during decision making time before starting an uptake.
- Facility Manager. Ensures that all equipment and laboratory facilities are set-up for test day. Ensures that facilities are properly cared for, and secured following use.

Nicholas Welschmeyer.

- Principal Investigator – Scientist. Ensures that the procedures are conducted in accordance with scientific protocols. Responsible for validating test parameters, and conducting validity discussions with the Verification Organization.

- Sampling Team Lead. Ensures that sampling system is properly operated, and that required volumes are collected and processed.
- Biological Analysis. Ensures that biology analysis team conducts assessments in accordance with protocols. Reviews and assists when problems and questions arise. Reviews preliminary data, conferring with VO if and as needed.
- Water Quality – Grab Samples. Ensures that discrete, grab, samples are obtained, handled, and sent to the appropriate laboratories (internal or external). Receives and analyzes reports.

Staff with Single Task Assignments.

- John Coyle. Lead Operator and Relief Chief Engineer of ship.
- Dan Lintz. Ballast System Operator and Chief Mate of ship.
- Brian Maurer. Phytoplankton Analysis and Facility Laboratory Lead.
- Jules Kuo. Zooplankton Analysis Lead.

6.2 Test Validation Parameters

The test validation parameters are provided in the Project Plan. These are duplicated here for convenience. Please refer to the Project Plan for a more detailed explanation of these parameters, actions to perform if one or more parameters are not met, and interpretation of levels and durations. The remainder of this section will list the baseline numbers without error bands or qualifiers for simplicity. However, the actual criteria is as listed in the Project Plan Valid Test Parameter Table.

Table 5 – Valid Test Parameters, One Land-Based Cycle

	Criteria		Uptake Events			Discharge Events		
			Treat 1	Treat 2	Control	Treat 1	Treat 2	Control
Treatment Line and Tanks								
Average (m³/hr)	250 ± 10%							
Volume at end Cycle (m³)	200 (-0%/+10%)							
Control Line and Tank								
Total Volume (m³)	200 (-0%/+10%)							
Combined Sample Volume (m³)								
Uptake	≥ 1							
Control Discharge	≥ 3							
Treatment Discharge	≥ 9							
Ballast Hold Duration (hours)	120 (-0%/+10%)							
Water Quality	Brackish	Salt						
Salinity (PSU)	10 - 20	>32						
Temperature (Celcius)	4 - 35	4 - 35						
DOC (mg/L)	≥ 6	≥ 6						
POC (mg/L)	≥ 5	≥ 4						
TSS (mg/L)	≥ 50	≥ 24						
Uptake Living Populations								
≥50 microns (organisms/m³)	10^5							
≥10 < 50 microns (organisms/mL)	10^3							
<10 microns (bacteria/mL)	10^4							
Control Living Populations								
≥50 microns (organisms/m³)	100							
≥10 < 50 microns (organisms/mL)	100							
<10 microns (bacteria/mL)	500							

6.3 Treatment System Operation

The BWTS includes a filtration system and a UV device. used on ballast and deballast ~~The filtration device is bypassed during de-ballasting.~~ The BWTS is controlled remotely by the Facility automation system. The automation system can place the BWTS in the following modes: Standby, Ballast, and De-Ballast. In addition, the BWTS control system provides the Facility automation system with alarm and fault status signals.

Standby

All operations start with the BWTS in Standby. The Standby mode is engaged by the “stop” command from the Facility automation system. This means that power is supplied to the BWTS allowing operation of the control system, electrical enclosure heaters, etc. All valves in the BWTS are automatically closed.

The BWTS can enter Standby from the Ballasting mode. In this case, the Facility selects Standby (“Stop” on the Facility user interface) and the BWTS automatically starts a backflush cycle, then enters a UV lamp cool-down period, and then closes all BWTS valves. Entering Standby from Deballasting is similar, but without the back flush step. The Facility will then close valves to isolate the BWTS.

Ballasting Operation

For a ballasting operation, the Facility will prime the BWTS inlet piping and bleed air at the BWTS inlet. The Facility will then place the BWTS into Ballast mode.

The BWTS then automatically undergoes its start-up procedure: opens the filter inlet valve, ensure the filter bypass valve is closed, and turns on the UV lamps. Once the UV lamps have reached operational output temperature, the BWTS will automatically open the outlet valve and allow fully treated water to pass through to the ballast tanks.

Back Flushing Cycle

At certain intervals, based on differential pressure, the BWTS will initiate a back flush cycle. In this timed cycle a percentage of inlet ballast water is use to back flush the filter elements with the effluent sent overboard to waste. The system is designed to deliver treated effluent at a constant rate, however delivery may be reduced during these back flush periods which could slow uptake. Ballast system pumping and sampling operations do not change during these periods.

De-Ballasting Operations

For a de-ballasting operation, the Facility will prime the BWTS inlet piping and bleed air at the BWTS inlet. The Facility will then place the BWTS into De-Ballast mode.

The BWTS then automatically undergoes its start-up procedure: ensures the filter inlet valve is closed, opens the filter bypass valve, and turns on the UV lamps. Once the UV lamps have reached operational output temperature, the BWTS will automatically open the outlet valve and allow fully treated water to pass through to the ballast tanks.

6.4 Pumping and Piping Operation

Test cycles are the ballasting uptake and discharge operations that specifically utilize the Treatment Tanks and Control Tank, and include efficacy testing to compare uptake and discharge organism counts for the Treatment Tanks and Control Tank.

The “Piping System Line-up Diagrams,” and the “Operation of BWTS and Facility Piping System” are located in the SOPs, which provide detailed instructions and logs for running the BWTS and Facility piping system. The following section outlines the testing procedures.

Flushing and Draining Prior to Testing

The piping system must be flushed with a mild bleach solution and then drained prior to a Land-Based cycle uptake.

Treatment Tank 1 Uptake

The objective of the uptake is to fill Treatment Tank 1 through the BWTS at a rate of 250 m³ +/-10% per hour until the Treatment Tank volume is not-less-than 200 cubic meters. Suction is taken from Source Tank 1.

The actual steps of the treatment uptake are detailed in the SOPs. A high level review is provided here.

- Piping system is arranged to take suction from the sea with the treatment pump, and deliver water to the BWTS inlet. Until the BWTS is running, the water recirculates through the piping system and is returned to the pump suction. or a ballast tank
- The treatment pump is started, and air is bled from the system.
- The BWTS is turned on and set to Ballast Mode. or recirculate via treatment and pump manifold
- Piping system is managed to be at the target flow.
- Once the BWTS is warmed up and online it will automatically open its outlet valve, allowing treated ballast water to flow through discharge piping to the overboard.
- The piping recirculation route is closed, ensuring all water flows through the BWTS to the overboard. or recirculated through BWTS and back to pump suction
- Flow rate is managed using the ballast treatment pump and piping system.
- Water Quality Monitors, providing continuous monitoring of the ballast water at the pump discharge, are placed online.
- The sampling system is flushed with raw seawater while suction continues to be taken from the sea and discharges to the overboard.
- Once the sampling system is flushed and ready to begin collection (at the same time):
 - The Tank 3-154-1 fill valves are opened, and the discharge to overboard is shut.
 - The Source Tanks valves are opened, and the seachest supply valve is shut.
 - Sampling begins by filling three tubs with inflowing water, and logging the start into the automation system.
- Flow rate management occurs as discussed above until the desired tank level is reached.
- Treatment uptake is completed by (at the same time):
 - Treatment Tank 1 is confirmed at a volume not-less-than 200 m³.
 - The BWTS is shutdown.
 - The treatment pump is stopped, and the Treatment Tank 1 fill line is immediately closed. This ensures that the BWTS remains flooded during its shutdown cycle.
 - All remaining valves left open are secured.
 - The BWTS shutdown cycle is completed.

Treatment Tank 2 Uptake

Treatment Tank 2 is filled in the same manner as Treatment Tank 1, at a rate of 250 m³ +/- 10% per hour until the Treatment Tank volume is not-less-than 200 cubic meters. Suction is now taken from Source Tank 2. Treatment uptake is completed by (at the same time):

- Treatment Tank 2 is confirmed at a volume not-less-than 200 m³.
- Discharge from the BWTS is sent from Treatment Tank 2 to the Control Tank.
- Treatment Tank 2 inlet piping is double blocked and bled.
- Sampling is switched so that sampling begins in the next set of tubs.

Control Tank Uptake

Following Treatment 2 uptake, the system is switched over to begin filling the Fore Peak Tank. This serves as the Control holding tank. The Control uptake continues to take water from Source Tank 2. After the switch is complete, the BWTS is shutdown and isolated. The Control Tank is filled to a volume not-less-than 200 cubic meters.

- Once BWTS discharge is switched to send water to the Control Tank, the 01 Deck by-pass valves are opened, and the BWTS is shutdown.
- Flow management occurs as discussed above.
- Once the BWTS is completely shutdown, it is isolated from the system.
- When Source Tank 2 reaches a level of 300 mm (1 ft), Source Tank 1 is opened, sending ballast water to the Control Tank, and Source Tank 2 is closed.
- Flow management occurs as discussed above.
- Control uptake is completed by (at the same time):
 - Control Tank is confirmed at a volume not-less-than 200 m³.
 - The treatment pump is stopped.
 - All remaining valves left open are secured.

Flushing and Draining Between Fill and Discharge

The piping system must be flushed with a mild bleach solution and then drained between the filling and discharging cycles to avoid contamination.

Treatment Tank 1 Recirculation and Discharge

Note: The Treatment Tanks must be discharged BEFORE the Control Tank.

Following a holding time not-less-than 120 hours, Treatment Tank 1 will be discharged overboard as detailed in the SOPs. The ballast water will be sampled in triplicate at the Main Deck sample port in accordance with the SOPs.

- The piping system is arranged to take suction from Treatment Tank 1 with the treatment pump, and deliver water to the BWTS inlet. Until the BWTS is running, the water recirculates directly to the pump suction.
- The treatment pump is started, and air is bled from the system.
- The BWTS is turned on and set to De-Ballast Mode.
- Flow management occurs as discussed above.

with filter backwash
returned to holding tank

- Once the BWTS is warmed up and online it will automatically open its outlet valve, allowing treated ballast water to recirculate. The direct pump recirculation route is closed.
- Flow management occurs as discussed above.
- Water Quality Monitors are placed online.
- The sampling system is flushed with treated ballast water while the system continues to recirculate water from Treatment Tank 1.
- Once the sampling system is flushed and ready to begin collection (at the same time):
 - The discharge is sent to overboard and the treated water recirculation route is closed.
 - Sampling begins by placing the nets in front of the flowing sample water, and logging the start into the automation system.
- Tank levels are monitored as follows:
 - Pumping rate of 250 m³/hr is maintained until tank water level reaches ~300 mm in height.
 - Pumping rate is decreased to 50 cubic meters per hour starting at 300 mm in height, until the pump loses suction.
- When the treatment pump loses suction, the BWTS and pump are secured.
 - The BWTS is shutdown.
 - The treatment pump is stopped and the piping to overboard is closed immediately. This ensures that the BWTS remains flooded during its shutdown cycle.
 - All remaining valves left open are secured.
- Sampling system flow will naturally stop, once the ballast pump loses suction and is no longer pumping ballast water. The sampling system inlet valves are then shut, and the sampling system secured.

Treatment Tank 2 Recirculation and Discharge

Note: The Treatment Tanks must be discharged BEFORE the Control Tank.

Treatment Tank 2 is discharged in the same manner as Treatment Tank 1.

Control Tank Recirculation and Discharge

The Control Tank is discharged after the Treatment Tanks. The discharge procedure is similar to those for the Treatment Tanks. The primary differences include:

- The BWTS is secured, and the 01 Deck by-pass is open.
- The sampling volumes required are less, therefore a different sampling manifold is used.

6.5 Sampling Procedures

These procedures are performed to provide samples to support the analyses described in Section 6.7 – Biological Efficacy Analysis. In addition, these samples are used for the “grab

sample” assays described in Section 6.6 – Water Quality Analysis. The below table, Sampling Procedure Coordination, matches the pumping system operation to a particular sampling procedure and the resulting sample replicates and volumes.

Table 6 - Sampling Procedure Coordination

Pumping Operations		Sampling Procedures					
	Rate** (m3/hr)	Name	Location	Pitot/ Size (inches)	Each Replicate		
					Rate** (lpm)	Volume	
						10 - 50 μ m	$\geq 50 \mu$ m
Uptake							
Treatment 1 Untreated	250	3 x 0.4	B1, B2, B3	S1 (1 x 1.05")	8	3 x 22 liters	3 x 0.4m3
Treatment 1 Treated	250	T-0	170L	S2 (1 x 0.58")	drip	170L	
Treatment 2 Untreated	250	3 x 0.4	B1, B2, B3	S1 (1 x 1.05")	8	3 x 22 liters	3 x 0.4m3
Treatment 2 Treated	250	T-0	170L	S2 (1 x 0.58")	drip	170L	
Control (Untreated)	250	3 x 0.4	A1, A2, A3	S1 (1 x 1.05")	8	3 x 22 liters	3 x 0.4m3
Discharge							
Treatment 1 Treated	250	3 x 3	A1, A2, A3	S4 (3 x 1.68")	75	3 x 22 liters	3 x 3m3
Treatment 2 Treated	250	3 x 3	A1, A2, A3	S4 (3 x 1.68")	75	3 x 22 liters	3 x 3m3
Control (Untreated)	250	3 x 1	B1, B2, B3	S2 (1 x 1.61")	25	3 x 22 liters	3 x 1m3

**During tank stripping pumping rates slows down.

Uptake water sample rate 22.3 lpm

Sampling Overview

Ballast water sampling procedures vary as per the ballasting operations as follows:

Ballast Water Uptake Untreated Water Samples (9 x 0.4 m3 and 9 x 22 liters).

During ballast water uptake, untreated water is taken from the Source Tanks and moved to Treatment Tank 1, then Control Tank, and then to Treatment Tank 2. At each of these three steps, three untreated samples of at least 0.4 cubic meters are taken before the BWTS. This method assures sampling during the entire uptake event, and produces three (3) triplicate samples for each uptake totaling in greater than one (1) cubic meter. From each uptake, a 22-88L carboy sample (based on biological density) will be taken from each 0.4 m3 sample, and each will be filtered for analysis of the $\geq 50 \mu$ m size class.

During each uptake a carboy of 22 liters is filled continuously, unfiltered, from each sample tub. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 μ m size class, for each uptake. A total of nine (9) 20 L samples for the 10 – 50 μ m size class are collected.

Ballast Water Uptake Treated Water Samples (2 x 170 liters).

During ballast water uptake to Treatment Tank 1 and Treatment Tank 2, 170 liters of treated water is sampled after the BWTS. The 170L sample is split into one-third volumes at the beginning, middle, and end of each uptake. This provides three (3) samples totaling in 170 liters for each uptake.

Ballast Water Discharge Treatment Tank 1 Samples (3 x 3m3 and 3 x 22 liters).

During ballast water discharge from Treatment Tank 1, integrated samples of treated water in triplicate of at least 3.0 cubic meters each are taken after the BWTS. This provides a total of three (3) 3.0 cubic meter samples for a total volume of at least 9.0 cubic meters. This method assures sampling during the entire discharge event for analysis of the $\geq 50 \mu$ m size class.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from each of the three same sample lines. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 µm size class. **Treated discharge backwash samples will be taken.**

Ballast Water Discharge Treatment Tank 2 Samples (3 x 3m³ and 3 x 22 liters).

These procedures are identical to Discharge Treatment Tank 1 Samples, producing three (3) 3.0 cubic meter samples for a total volume of at least 9.0 cubic meters for analysis of the ≥50 µm size class and three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 µm size class. **Backwash samples will be evaluated for organism count.**

Ballast Water Discharge Control Samples (3 x 1m³).

During ballast water discharge from Control Tank, integrated samples of treated water are taken in triplicate of at least 1.0 cubic meters each. This provides a total of three (3) 1.0 cubic meter samples for a total volume of at least 3.0 cubic meters. This method assures sampling during the entire discharge event for analysis of the ≥50 µm size class.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from each of the three same sample lines. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 µm size class.

Sampling pitots for each ballasting event are sized to maintain a consistent fluid velocity, despite different flow rates designated to event-specific sampling procedures.

Sampling Coordination

Ballast water uptake includes one event that uptakes to Treatment Tank 1 (3-154-1) and then the Control Tank (6-E-0), and a second event that uptakes to Treatment Tank 2 (3-154-2). This sequence allows the one Control Tank to serve both Treatment Tanks.

The first uptake event primes the BWTS inlet piping, starts the BWTS, and passes ballast water from the seachest through the BWTS and then overboard (sea-to-sea). The sea-to-sea mode enables the sampling team to set-up the sampling system. The pumping team then: switches suction to the Source Tanks, fills Treatment Tank 1 with treated water, and switches to start fill of the Control Tank while shutting down the BWTS. Pumping is then stopped to clean the piping system of control ballast water. After the brief stop for cleaning, Treatment Tank 2 is filled in the same manner as Treatment Tank 1.

Ballast water discharge proceeds after ballast water is held for not-less-than 120 hours. Discharge includes three events that discharge Treatment Tank 1 (3-154-1), Treatment Tank 2 (3-154-2), and Control Tank (6-E-0).

The first discharge event primes the BWTS inlet piping, starts the BWTS, and takes suction from Treatment Tank 1. The treated water passes the BWTS, and returns to the treatment pump suction (recirculation). The recirculation mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge to overboard, emptying Treatment Tank 1 to sea, while the sampling team takes triplicate samples of the treated water. In the second event, Treatment Tank 2 is discharged in the same manner as Treatment Tank 1. The Control Tank is then recirculated, by-passing the BWTS. The untreated control water is then sampled and discharged to sea.

Communications between the pumping team (opening and closing valves, operating the treatment system pump, and operating the BWTS) and the sampling team is performed by radio, visual communications, and the automation system ballast order telegraph (BOT). Of these, the BOT is the primary and definitive means of communication.

The sampling team will prepare the sampling system by flushing all equipment, hoses, and other devices with raw seawater before uptake or held ballasted before discharge. During set-up, the sampling system will also balance flows into the tubs where the plankton nets are rigged. However, the sampling team will only send ballast water through the plankton nets when directed by the pumping team. This is called “netting.” Only during an actual uptake or discharge are the samples netted, in order to maximize the sample representativeness of the ballast tank contents.

6.6 Water Quality Analysis

Continuous Data

In addition to collecting site-specific data, as detailed in the *Facility Preparation* section, Water properties passing the Facility pump will be continuously monitored by the Sea Bird TSG, including temperature, conductivity, salinity, dissolved oxygen, transmissivity, and chlorophyll florescence. These parameters will be logged to the individual instrument, as well as collected and stored in the automation system.

Table 7 – Continuous Monitoring Parameters

Sensor	Maker	Type	Calibration Procedure	Reporting Units	Range
Temperature	Sea-Bird Electronics	SBE 21	Nominally 1/year	degrees C	-5 to +35
Conductivity	Sea-Bird Electronics	SBE 21	Nominally 1/year	S/m	0 to 7
Transmissometer (25 cm)	Wet Labs	C-Star	Nominally 1/year	% transmittance or beam attenuation	0-100
Dissolved Oxygen	Sea-Bird Electronics	SBE 43	Nominally 2/year	mg/L	120% of saturation
Fluorometer	Turner Designs	Cyclops-7	In-house, before each experimental run	ug/L	0.1 to 50

In-Situ Probe Measurements

A handheld hydrographic field meter will be used to measure dissolved oxygen, salinity and temperature in the biological sampling tanks at the time of sample collection. A bench top pH meter with Ross combination electrode will be used to measure pH. The measurements made here are redundant with those collected continuously by independent sensors associated with the GBF continuous data system.

Grab Samples

Challenge water samples will be taken at the time of ballast uptake to define empirically the chemical and biological characteristics of water entering the control and treatment ballast tanks. These samples will be drawn directly from the uptake pipe flow at the biological sampling station onboard *Golden Bear* using unsieved water, collected in triplicate and with the time-integrated method as described above. Challenge water living organism concentrations will be determined from sieved and unsieved water collections, as described in the above Sampling Procedures – Section 6.5. Table 8 provides sample listings with defined methods. Challenge water sample analyses will include the following:

- Salinity
- Temperature
- pH
- Dissolved Oxygen
- Total Suspended Solids
- Transmittance
- Particulate Organic Carbon
- Dissolved Organic Carbon
- Chlorophyll *a*

Table 8. Challenge water sample parameters and processing details

Parameter	Sample method/ volume	Processing	Sample Storage	Analysis	Notes
Salinity	YSI 6600 handheld probe	immediate	NA	Meter readings, handheld probe unit	Calibrated with Thermo-Fisher conductivity standards
Temperature	YSI 6600 handheld probe	immediate	NA	Meter readings, handheld probe unit	Factory calibrated thermistor probe
Dissolved oxygen	YSI 6600 handheld probe	immediate	NA	Meter readings, handheld probe unit	Calibrated with 100% air saturated water
pH	135 mL polypropylene bottle	Within 6 h of collection	Room °C	Beckman Model 70 pH meter	Two-point pH standardization
Total Suspended Solids (TSS)	2 L polypropylene bottle	Volumetric filtration onto pre-weighed 0.5 µm membrane filter	Dried at 85 °C for 3 hours, stored in vacuum dessicator @ room °C, analyzed within 30 d	Gravimetric weight determination after drying to constant weight, ± 0.1 mg	Weighed on 5-digit balance, granite weighing table (MLML)
Transmittance	20 mL acid-washed glass vial	Water, unfiltered, directly into glass sample container for storage	Room °C	Light propagation analysis to determine beam transmittance.	Readings from 370 nm to 650 nm, at 10 cm and 25 cm.
Particulate Organic Carbon (POC)	2 L polypropylene bottle	Volumetric filtration onto pre-combusted GF/F filter	Dried at 65 °C for 48 h, stored in vacuum dessicator@ room °C, analyzed within 30 d	CHN combustion analysis	Combustion analysis on CEC 440 CHN Analyzer (MLML)
Dissolved Organic Carbon (DOC)	20 mL acid-washed glass vial	Water passed through GF/F filter, directly into glass sample container for storage	Frozen -20 °C, analyzed within 30 d	Catalytic oxidation	Contract analysis with McCampbell Analytical, Inc. (EPA approved), Pittsburg, CA
Chlorophyll a	1 L amber polypropylene bottle	Volumetrically harvested onto 25 mm GF/F filter, immediately extracted in 1.2 mL 90% acetone	90% acetone extracts stored @ -20 °C, analyzed within 30 d	Single-step fluorometric assay for chl <i>a</i> ; C-8 HPLC for chlorophylls and carotenoids	Turner TD-700 filter fluorometer, calibrated with HPLC-purified authentic chl <i>a</i> standard (Welschmeyer 1990)

6.7 Biological Efficacy Analysis

Biological test procedures for determination of BWTS performance are detailed in this section of the QAPP. Biological testing methods are evolving continuously. There are few routine, time-tested methods for plankton viability determination at this time, such as *Standard Methods*, US EPA, ASTM, or otherwise. Therefore, methods must be devised to meet the quantitative challenges of regulatory performance standards. Detailed biological assays are included in the Standard Operating Procedures (SOPs). The biological efficacy methods detailed here reflect our desire to:

- Achieve the objectives of the Land-Based study as stated in the Project Plan.
- Utilize instrumentation and facilities at GBF that were assembled specifically for the purpose of viability testing, including development of new methodologies.

The tests for biological efficacy provide numeric counts of living organisms defined either by size category or specific pathogen. Methods for viable organism counts to be used by GBF scientists are detailed below, according to size and pathogen classification. Table 3 summarizes the critical measurements of organism viability made in this study, along with notes on methods and processing techniques.

Section 6.5 describes the collection of samples for these analyses.

Organisms Larger than 50 μm

Organisms larger than 50 μm in minimum physical dimension will be collected with Nitex mesh having a nominal pore size of 50 μm , measured on the diagonal (e.g., 35 μm Nitex plankton nets). Counts of live and dead organisms larger than 50 μm will be made using the so-called ‘poke and probe’ method under 30x (nominal) dissecting microscope observation (see below).

Organisms larger than 50 μm will be collected at each of three replicate sampling stations mounted on the Main Deck of *T/S Golden Bear*. Each sampling station provides metered flow into the mouth of the zooplankton net (three simultaneous replicates). The nets are positioned within large polyethylene containers that retain filtrate water to a user-controlled height, thus bathing the zooplankton net in surrounding water to reduce damage to live captured organisms. Sample water from the appropriate source (e.g., Treatment Uptake, Control Uptake, Treatment Discharge, Control Discharge) will be directed through each net at uniformly metered flow rates to yield continuous samples, integrated over the each ballasting operation. Three sequential 1 m^3 samples will be collected for uptake sampling. Three replicate 3 m^3 samples will be collected for each treatment discharge sampling, while three replicate 1 m^3 samples will be collected for control discharge sampling. The larger collection volume for treatment discharge attempts to improve statistical confidence in samples where the total number of live organisms is expected to be low.

Rates of ca. 25 L/min per each replicate will provide adequate flow to collect zooplankton samples of at least 1 m^3 each during the uptake and control discharge events. The sample flow rate will be increased by a factor of 3x for treatment discharge sampling; output from the calibrated flow meters will be logged continuously by the GBF data acquisition system. Each replicate sampling station will be fit with two nets: one to collect the bulk of the sample, and

a spare that can be quickly positioned in-line in case clogging becomes a problem. GBF's experience indicates that all sample volumes of 1 m³ will be easily handled by a single net. The large volume treatment tank samples will likely be accommodated by a single zooplankton net, since they will have been filtered (larger than 50 µm) by the BWTS.

Concentrated organisms will be immediately transported to the ship's laboratory for viability determination by microscopy. The samples will be maintained at ambient water temperature in a darkened, insulated container that is plumbed with flowing surface seawater from the ship. Cod end contents of the sample nets will be adjusted quantitatively to 500 mL with filtered seawater (0.7 µm GF/F filtrate) and counted immediately. Sample aliquots of 10 mL will be placed in a serpentine counting tray and observed under a stereo microscope; nominally, at ca. 30x magnification for determination of live/dead counts.

All efforts will be made to enumerate live organisms quickly, since the primary metric in ballast treatment performance tests is the determination of live organism counts. Live organisms will be counted first, followed by enumeration of dead organisms in the same sample aliquot. Animals will be designated as 'live' if they are fully intact and actively moving, exhibited an escape response when probed with a fine needle, or showed any internal/external movement. Organisms will be recorded as 'dead' if no activity or movement of any kind was observed, or if organisms were not intact. This viability determination is commonly referred as the 'poke and probe' technique. Totals and fractional portions of live and dead organisms will be tabulated, and all assays will be manipulated volumetrically (if required) so that organism concentrations (number/volume) can be reported. Two separate archive samples will be preserved in 4% buffered formalin, to allow for the inspection of general taxonomic diversity under less pressing time constraints, and kept for at least six (6) months.

Organisms Larger than 10 µm, but Smaller than 50 µm – Required Assays

It is widely recognized that the absolute numerical determination of viable unicellular protists in the size range 10 µm to 50 µm is ambiguous (Dobroski 2009). This follows from the fact that visible signs in distinguishing live from dead protists are usually not evident (except in the case of motile representatives). For this reason, several corroborative methods will be used on board ship to evaluate the diverse assemblage of microbiota occurring in the size range larger than 10 µm but smaller than 50 µm. The methods will include techniques that specifically yield estimates of viable cell concentrations. Whole water (unsieved) for use in preparing the 10 to 50 µm samples will be collected in 20 L carboys, as described in Section 3.

GBF will use a size fractionation technique to isolate the organisms smaller than 50 µm, but larger than 10 µm for all assays listed below. This is easily accomplished by concentrating the smaller than 50 µm filtrate (passed through a 50 µm sieve) on to custom made Nitex filters of 10 µm pore size on the diagonal (Nitex product 03/7-2) and re-suspending the retained particles for direct use in the assays below. GBF has been able to concentrate samples ten to one hundred times greater than ambient concentrations utilizing this method; this significantly increases the analytical sensitivity and precision of techniques used to measure low concentrations of viable organisms expected in treatment samples. Two separate archive samples will be preserved in 1% glutaraldehyde, to allow for the inspection of general taxonomic diversity under less pressing time constraints, and kept for at least six (6) months.

Chlorophyll-based most probable number (MPN) determination of viable cell concentration – Required Assay

Photoautotrophic growth will be measured from long term incubations (14d) by using whole-cell chlorophyll fluorescence analysis as a sensitive indicator of cellular growth. MPN culture arrays of serially-diluted sample water will be prepared with F/2 seawater media (adjusted for ballast water salinity) in clear micro-tubes (0.5 mL volume) that can be read directly in a Spex Fluoromax 2 spectrofluorometer on board ship. The MPN array will be constructed to yield optimized detection of 10 living organisms per mL (Woomer et al., 1990). Nominally the MPN culture arrays will include 25 tubes per sample (5 replicates x 5 dilution levels). MPN cultures will be maintained in an illuminated incubator at 5°C above natural temperature (to promote rapid growth) and monitored every other day for fluorometric indication of chlorophyll growth, defined as a two-fold increase in chlorophyll fluorescence relative to values scored at time zero. Triplicate MPN arrays will be set up for each sample, thus dictating the use of small growth tubes to conserve space within the incubator for all samples acquired from the three separate ballasting test cycles described here. One scientific technician will remain onboard ship after the last ballast test cycle to complete the daily fluorometric analysis of MPN arrays through the full 14d incubation period for each sample.

C-14 Primary Production Experiments – Required Assay

The radiotracer C-14 technique will be applied to uptake, control and treatment samples (each in triplicate) to yield physiological measurements of photosynthesis (carbon fixation rates). Experiments will be initiated and terminated at MLML in a walk in cold room nominally held at 13 °C. Samples will be prepared in triplicate, acid-washed polycarbonate bottles (125 mL), inoculated with C-14 (2 µCi) and incubated for 24 hours under continuous, constant illumination provided by high intensity LED lamps; bottles will be rotated continuously on motorized plankton wheels to ensure uniform irradiance exposure and to prevent settling of cells. C-14 processing will follow that of Welschmeyer et al. (1993). Whole water sample aliquots will be harvested onto GF/F filters (0.7 µm) and 10 µm nylon filters to estimate total and >10 µm photosynthetic rates, respectively (µgC L⁻¹ d⁻¹). Total dissolved inorganic carbonate (DIC) will be determined on a UIC CM5012 CO₂ Coulometer for proper determination of DIC specific activity (dpm/gC). Chlorophyll specific photosynthetic rates will be computed from Chl measurements made on the same water samples.

Organisms Larger than 10 µm, but Smaller than 50 µm – Corroborative Assays

The previously described *Required Assays* are complete for the 10 – 50 µm size class as specified by the IMO G8 Guidelines that this test plan must comply with. This test plan is not required to comply with the newly released ETV Protocol that includes additional testing requirements for the 10 – 50 µm size class. As such, these additional tests are listed here as optional Corroborative Assays.

Whole water (unsieved) for use in preparing the 10 to 50 µm samples will be collected in 20 L carboys, as described in Section 3.

It is hoped that the use of independent and corroborative methods will add confidence in the efficacy testing for the problematic 10 to 50 µm size class.

Flow cytometric analysis of live cells utilizing fluorescein diacetate (FDA) vital stain – Corroborative Assay

GBF will maintain a Becton-Dickinson FACScan flow cytometer on board ship throughout the two-three month experimental period to be used for quantitative analysis of living cell concentrations utilizing FDA vital stain protocol (Geary et al 1997; Hayakawa et al. 2008). FDA is a colorless reagent that freely passes through cell membranes and, when acted upon by living cellular esterase activity, is converted to the brilliant green fluorescent product, fluorescein, which readily marks viable cells for flow cytometric detection. GBF will focus primarily on the detection of larger phytoplankton cells, as opposed to colorless heterotrophs, since phytoplankton provide natural red chlorophyll fluorescence; this yields a robust, two-color discrimination (red/green) for quantitative cytometry. The cytometric technique depends on the detection of obvious cell populations using optical scattering and fluorescence signals. GBF will rely on natural 'red' chlorophyll fluorescence to determine natural phytoplankton population targets, as this provides the optimal optical discrimination to identify the 'green' fluorescent live cells after the addition of FDA. Inert fluorescent bead standards of 10 μm and 50 μm will be used to roughly establish the cytometric region of analysis (based on forward scatter), thus allowing us to gate out the more numerous, small cells (smaller than 10 μm) that are sure to be present in all samples.

Visual epifluorescence detection of viable 10 to 50 μm cells utilizing FDA and CMFDA tracers – Corroborative Assay

Visual enumeration of live cells in the 10 to 50 μm size category will be made using similar protocol to that described for flow cytometry (4.2.2.2). In this case, two fluorescent markers, FDA (fluorescein diacetate) and CMFDA (chloromethylfluorescein diacetate), will be applied simultaneously to maximize visual fluorescent signals and to minimize color fade during the counting procedure. FDA and CMFDA will be added to a final concentration of 5 μM and 2.5 μM , respectively, and incubated for 10 minutes before mounting in a covered 1 mL counting chamber for epifluorescence enumeration utilizing blue excitation and green emission.

Thus, three methods for enumerating viable cells in the size class smaller than 50 μm but larger than 10 μm will be used:

- MPN culture by chlorophyll detection.
- Viability staining with FDA, flow cytometric detection.
- Viability staining with FDA and CMFDA.

It is hoped that the use of independent and corroborative methods will add confidence in the efficacy testing for the problematic 10 to 50 μm size class.

Organisms Less than 10 μm

Five assays will be used in the detection of living organisms in the smallest size class, less than 10 μm . The first is the bulk assay for total cultivable bacteria and the remaining four assays are directed to specific microbial pathogens, *Escherichia coli*, *Enterococci sp.*, *Vibrio cholerae* serotype 01, and *V. cholerae* serotype 0139.

Bulk heterotrophic bacteria plate counts

Traditional sterile plating technique will be used to enumerate colony forming units (CFU) of the bulk bacterial community passing through a 10 µm sieve. Volumetric (100uL) sample aliquots (in triplicate) will be spread on sterile marine agar plates (Difco, 100 mm dia.) and incubated overnight (maximum of 24 hours) under dark conditions and at room temperature. Plates will be photographed in a digital image analyzer (Bio Rad Fluor S-Max) and enumerated using colony-counting software provided with the instrument. Data will be tabulated as CFU/mL.

Microbial pathogens

Test kits based on quantitative colony forming unit (CFU) measurements specific to *E. coli* and *Enterococci* will be used on board ship using sterile protocol. *E. coli* and *Enterococci* will be assayed using the Colilert[®] and Enterolert[®] test kits (Idexx, Inc.), which are based on MPN methodology and species-specific chromogenic reactions. Both assays will be prepared in triplicate using heat sealed dilution trays (Quanti-Tray[®] Idexx, Inc.) incubated at 35 °C for 24 h. *V. cholera* will be assayed using test kits for the 01 and 0139 serotypes, Cholera Smart[®] II and Bengal Smart[®] II, respectively (New Horizons Diagnostics, Inc.). The kits were originally produced for the purpose of obtaining quick (20 min) Cholera presence/absence tests in fecal stool samples; this method is rapid, but relatively insensitive. We have worked with New Horizons (Larry Loomis, CEO) to show that tolerance limits for Cholera of less than 1 CFU/100 mL can be achieved with a 48 h, 35°C incubation using Cholera Smart kits as packaged by the manufacturer (determined from quantitative dilutions of actively growing cultures of *V. cholerae* 01 and 0139 serotypes). GBF will use the prolonged incubation for *V. cholera* to achieve positive/negative scores at less than 1 CFU mL. All of the microbial assays above will be completed in triplicate, and all waste solutions will be bleach-sterilized before disposal.

Section 7 Water Toxicity Testing

Overview

Samples will be collected for the purpose of toxicity testing during a treatment discharge and a control discharge. Pacific EcoRisk (PER) has been contracted to perform this work. One toxicity sampling event will occur during brackish testing, and one sampling event will occur during marine testing. Samples for toxicity testing will be collected concurrently with biological efficacy sampling. No biological efficacy analysis will be performed on the toxicity testing samples.

Sample Collection

A representative from PER will collect the toxicity testing samples from a sample port downstream of the ballast water sample tubs. For treatment discharge, a total volume of 150 liters will be collected for toxicity testing during each sampling event. Water will be collected near the beginning, middle, and end of the treatment discharge. 50 liters will be collected approximately 5 minutes after the discharge begins, 50 liters will be collected approximately 20 minutes into the discharge, and 50 liters will be collected approximately 40 minutes into the discharge. All sample water will then be mixed (at the PER laboratory under controlled conditions) to make a composite sample that is representative of the entire treatment tank discharge.

For control discharge, a total volume of 75 liters will be collected for toxicity testing during each sampling event. Water will be collected near the beginning, middle, and end of the control tank discharge. 25 liters will be collected approximately 5 minutes after the discharge begins, 25 liters will be collected approximately 20 minutes into the discharge, and 25 liters will be collected approximately 40 minutes into the discharge. All sample water will then be mixed (at the PER laboratory under controlled conditions) to make a composite sample that is representative of the entire control tank discharge.

Toxicity Testing

Toxicity testing will be performed in accordance with BWTS representative requirements and as approved by the Verification Organization. Both acute and chronic toxicity testing will be performed on the discharge samples. Three taxonomic groups will be evaluated: algae, crustaceans, and fish.

Refer to the *Toxicity Study Design* and *Summary of Toxicity Test Conditions* tables prepared by PER that are included in Appendices B and C, respectively.

Reporting

A report containing the test results will be prepared by PER and provided directly to a BWTS representative.

Section 8 Assessments

As per the Project Plan, the Verification Organization for this project will audit project documentation and performance for compliance with the TQAP and the IMO G8 Guidelines.

Appendix A Project Training Requirements

Quality is responsible to ensure that each person is trained as appropriate to their tasks on the project, as follows:

- Facility Training:** This training covers all information provided in the TQAP, along with specialized training relevant to their role per the test plan; i.e., Confined Spaced Entry Procedures and Documentation, Safety Reviews, etc.
- Equipment Training:** This training covers all information provided by the equipment manufacturer, along with specialized training relevant to the role in testing; i.e., System Installation Requirements Review (particularly US Coast Guard and ABS requirements for electrical connections and ground protection), Lock-Out/Tag-Out, etc.
- SOP Training:** This training ensures that personnel can successfully complete their assigned tasks using the appropriate SOP.

Quality is also responsible for documenting and maintaining training program records. The Director, Lead Operator and Lead Scientist will conduct the training unless otherwise designated by Quality. The logs on the following pages are for documentation of the project training completed by all GBF personnel before the start of testing. A copy of these training records will be filed on board the GBF, and a summary will be included in the final testing report.

Training Logs

Ballast Facility: William Davidson is the chief engineer of the *Training Ship GOLDEN BEAR* and has been the lead engineer during the construction of the GBF and has become expert with all of the technical aspects of the system. Bill is the trainer for those GBF personnel who operate the pumps, valves, etc.

List of personnel trained to operate the ballast pump and valve system:

- Dan Lintz, trained November 2010
- John Coyle, trained November 2010
- Dan Weinstock, trained May 2010 and updated November 2010
- David Coleman, trained May 2010 and updated November 2010
- Bill Schmidt, trained July 2010 and updated November 2010

Water Quality: Richard Muller has installed and maintained in situ water quality sensors for a good part of his career as a technician onboard various oceanographic research vessels during the past 20 years. His training has been primarily through manufacturer interaction and at-sea experience troubleshooting instrumentation.

Biological:

Dr. Nicholas Welschmeyer is professor and research scientist at the Moss Landing Marine Laboratories and has performed research on ballast water and ballast treatment systems for the past seven years with 31 years of field experience in biological oceanography. All biological analysts are trained within the laboratory of Nicholas Welschmeyer. First they are given the full set of standard operating procedures verifying that they have a command of the techniques. Second, for subjective analyses, analysts count trial samples simultaneously to determine comparability between analysts. Third, all biological analysts are familiarized with instrumentation used for ETV testing.

List of scientific technicians are:

- Erin Jensen
- Julie Kuo
- Brian Maurer
- Jeff Johnsen

Log Sheet 1: Facility Training

[illegible]

Log Sheet 2: Equipment Training

[illegible]

Log Sheet 3: SOP Training

[illegible]

Appendix B Toxicity Study Design

Andrew Daley
TrojanUV
3020 Gore Road
London, Ontario N5V 4T7

March 23, 2012

Dear Mr. Daley:

At the request of TrojanUV, Pacific EcoRisk has prepared a toxicity testing study for evaluating the toxicity of a TrojanUV ballast water treatment system installed on the Golden Bear Facility in San Francisco Bay. The study described below is design to meet the regulatory requirements of Det Norske Veritas (DNV-Norway).

Toxicity Testing Facility

Pacific EcoRisk (PER) is an environmental consulting firm conducting research and testing in the fields of environmental toxicology, aquatic biology, and environmental chemistry. PER has been in business since 1994, and is the only California laboratory that is nationally accredited through the National Environmental Laboratory Accreditation Program (NELAP). Although the testing will not be performed under GLP standards, the testing will be performed under US standards via NELAP requirements, which includes standard operating procedures, stringent QA measures, raw data archives, and rigorous staff training. PER's applies the NELAP principles with the goal of to producing high-quality, cost-effective, and often innovative solutions to complex environmental problems. PER has a broad range of experience in a variety of regulatory programs, and has performed toxicity testing for a wide range of clients, which include local, state, and federal regulatory agencies, industry and agriculture, ports and marinas, maritime industry, POTWs, US military services, as well as support services for other environmental or engineering firms. PER's state-of-the-art ~15,000 ft² facility readily has the capacity to complete ~5,000 toxicity tests performed each year, with high-quality testing being the focus.

Project Management and Staff:

An important part of PER's success in evaluating environmental issues is the knowledge, skills, and expertise provided by the team of scientists comprising the PER staff. PER's senior management team scientists (Dr. Scott Ogle, Jeffrey Cotsifas, and Stephen Clark) have all earned degrees (B.S. to Ph.D.) specializing in aquatic toxicology, with over 75 years of combined experience in the field. The management team members have made significant contributions to the field of aquatic ecotoxicology. Each senior manager is knowledgeable regarding the need to integrate the science within the regulatory framework, and each has been charged with the task

of helping PER clients understand how to answer regulatory questions and to address regulatory requirements. Stephen Clark will serve as the TrojanUV Project Manager, and will work closely with Marcie Merksamer of EnviroManagement to meet all project needs.

PER's supporting staff is comprised of 16 degreed scientists hold either B.S. or M.S. degrees, and have a reputation for high quality work and 17 technicians, many of whom hold degrees, and 3 administrative support staff. The staff are uniquely qualified to not only work on routine compliance monitoring needs (e.g., NPDES, ballast water testing, etc.), but also have exceptional expertise at the mid- and upper-level of our company that allows PER to be successful in working on challenging toxicity issues for their clients (e.g., TIEs/TREs, water effects ratios/site specific standards, biological community impacts, etc.). All staff participating in the testing of TrojanUV samples will have met a demonstration of capability for the test species involved and will have demonstrated acceptable ongoing performance of the test methods. Mike McElroy, a PER Senior Scientist, will serve as the Project Lead for TrojanUV. Mike has ~10 years of testing experience, including several years of managing ballast water testing studies.

Proposed Toxicity Study Design

Both acute and chronic toxicity testing will be performed on TrojanUV samples collected to model both an estuarine discharge (i.e., low salinity) and marine discharge (i.e., high salinity) scenario. The recommended testing protocols are presented in the table below.

Toxicity Testing Protocols for TrojanUV Ballast Water Testing				
Species	Endpoints	Duration	Acute/Chronic	Method/Manual
<i>Mysidopsis bahia</i>	Survival	48-hour	Acute	EPA 821-R-02-012
<i>Menidia beryllina</i>	Survival	96-hour	Acute	EPA 821-R-02-012
<i>Thalassiosira pseudonana</i>	Growth	96-hour	Chronic	ASTM 1218-04
<i>Mysidopsis bahia</i>	Survival and Growth	7-day	Chronic	EPA 821-R-02-014 (Method 1007.0)
<i>Menidia beryllina</i>	Survival and Growth	7-day	Chronic	EPA 821-R-02-014 (Method 1006.0)

A PER scientist will collect the samples on the Golden Bear Facility. The scientist will work with Golden Bear Facility staff to identify the proper collection point for the ballast water treatment sample and ballast control tank sample; each sample will be collected once for each testing event, with a sufficient volume collected for all solution renewals required for each test. All samples will be transported back to the PER laboratory, on ice and under chain of custody, within ~30 minutes of final sample collection. Given that multiple sample containers will be needed during sample collection, the samples will be composited back at the PER laboratory and then placed back into the original sample containers. This will assure that there is no difference between the sample containers. All samples are stored in the dark at 0-6 °C when not being used for solution preparation.



Each acute and chronic toxicity test will be performed with the following dilution series: 0%, 6.25%, 12.5%, 25%, 50%, and 100% ballast water. Appropriate Lab Control Water designated for each test method will be used as the diluent. A Ballast Water Control Tank sample will also be tested at 100% solution. As a measure to understand the sensitivity of each batch to test organisms used for the testing, concurrent reference toxicant tests will be performed for each test species and method.

Study Schedule

The estuarine discharge event is scheduled for Monday, April 2, and the marine discharge event is scheduled for Monday, April 16. Toxicity tests will be initiated within the 36-hour sample holding time limit identified in the EPA and ASTM testing manuals. An email summary of the test results will be provided to TrojanUV ~ 9 days after test initiation, when the growth data will be available and any required statistical analyses have been performed. A pdf report will be provided within 10 business days of the termination of the chronic toxicity tests.

If you have any questions regarding this literature search, please feel free to contact my colleague Dr. Scott Ogle or myself at (707) 207-7760.

Sincerely,

Stephen L. Clark
Vice President/Special Projects Director

Appendix C Summary of Toxicity Test Conditions

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE MYSID, <i>Americamysis bahia</i> (FORMERLY <i>Mysidopsis bahia</i>), ACUTE TOXICITY TEST OF BALLAST WATER DISCHARGE (EPA-821-R-02-012).	
1. Test type	Static non-renewal
2. Test duration	48 hours
3. Temperature	20°C ±1°C; or 25°C ±1°C
4. Light quality	Ambient laboratory illumination
5. Light intensity	50-100 ft-c (10-20 µE/m ² /s)
6. Photoperiod	16-h light: 8-h darkness
7. Test chamber size	250-mL
8. Test solution volume	200-mL
9. Renewal of test solutions	None
10. Age of test organisms	1-5 day juveniles with ≤ 24-hour range in age.
11. No. organisms per test chamber	10 (minimum)
12. No. of replicate chambers per concentration	4 (minimum)
13. No. organisms per concentration	40 (minimum)
14. Feeding regime	Prior to test initiation and once daily. 0.2 mL <i>Artemia</i> nauplii concentrate (~100 per mysid).
15. Test chamber cleaning	Cleaning not required
16. Test solution aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17. Dilution water	Type 1 water salted up with Crystal Sea bioassay-grade salts. Salinity: 32-34ppt
18. Test concentrations	Ballast Water sample: 6.25%, 12.5%, 25%, 50%, and 100%, a Lab Water Control, and a ballast water control (from ballast water control tank).
19. Endpoint	Percent survival (mortality)
20. Sample and sample holding requirements	Grab or composite sample first used within 36 h of of sample collection.
21. Sample volume required	2 L
22. Test acceptability criteria	90% or greater survival in controls

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR
SILVERSIDE, *Menidia beryllina*, ACUTE TOXICITY TEST OF BALLAST WATER
DISCHARGE (EPA-821-R-02-012).

1. Test type	Static-renewal
2. Test duration	96 hours
3. Temperature	20°C ±1°C; or 25°C ±1°C
4. Light quality	Ambient laboratory illumination
5. Light intensity	50-100 ft-c (10-20 µE/m ² /s)
6. Photoperiod	16-h light: 8-h darkness
7. Test chamber size	600-mL (250-mL, minimum)
8. Test solution volume	400 mL (200 mL, minimum)
9. Renewal of test solutions	After 48 h
10. Age of test organisms	9-14 day juveniles with ≤ 24-hour range in age.
11. No. organisms per test chamber	10 (minimum)
12. No. of replicate chambers per concentration	4 (minimum)
13. No. organisms per concentration	40 (minimum)
14. Feeding regime	2 hours prior to test initiation and 48-h renewal. 0.2 mL <i>Artemia</i> nauplii concentrate.
15. Test chamber cleaning	Cleaning not required during test
16. Test solution aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17. Dilution water	Filtered (1 µm) seawater or Type 1 water salted up with Crystal Sea bioassay-grade salts. 1-32 ppt – <i>M. beryllina</i> 15-32 ppt – <i>M. menidia</i> & <i>M. peninsulae</i>
18. Test concentrations	Ballast Water sample: 6.25%, 12.5%, 25%, 50%, and 100%, a Lab Water Control, and a ballast water control (from ballast water control tank).
19. Endpoint	Percent survival (mortality)
20. Sample and sample holding requirements	Grab or composite sample first used within 36 h of completion of sample collection.
21. Sample volume required	3.1 L per renewal day
22. Test acceptability criteria	90% or greater survival in controls

**SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR
ALGAL (*Thalassiosira pseudonana*) GROWTH TEST OF BALLAST WATER DISCHARGE
(ASTM E1218-04)**

1. Test type	Static
2. Test duration	96 hours
3. Temperature	20 ± 2°C
4. Light quality	“Cool white” fluorescent lighting
5. Light intensity	82-90 $\mu\text{E}/\text{m}^2/\text{s}$
6. Photoperiod	Continuous illumination
7. Test chamber size	250 mL
8. Test solution volume	100 mL
9. Renewal of test solutions	None
10. Age of test organisms	4-7 days old
11. Initial cell density	20,000 cells/mL
12. # of replicates chambers per concentration	Four
13. Shaking rate	Once daily by hand
14. Test chamber cleaning	Rinsed with dilution water. None during test.
15. Dilution water	0.45 μm filtered seawater or Type 1 water salted up with Crystal Sea bioassay-grade salts.
16. Dilution factor	Ballast Water sample: 6.25%, 12.5%, 25%, 50%, and 100%, a Lab Water Control, and a ballast water control (from ballast water control tank).
17. Test endpoint	Growth
18. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours of sample collection.
19. Sample volume required	2 Liters
20. Test acceptability	1 x 10 ⁵ cells/mL in controls

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSID, <i>Americamysis bahia</i> (FORMERLY <i>Mysidopsis bahia</i>), LARVAL SURVIVAL AND GROWTH TEST OF BALLAST WATER DISCHARGE (EPA-821-R-02-014, EPA Test Method 1007.0)	
1. Test type	Static renewal
2. Test duration	7 days
3. Salinity	20 to 30 ppt \pm 2 ppt
4. Temperature	26 \pm 1°C
5. Light quality	Ambient laboratory illumination
6. Light intensity	50-100 ft-c (10-20 μ E/m ² /s)
7. Photoperiod	16 hrs light:8 hrs darkness
8. Test chamber size	400-mL
9. Test solution volume	200 mL
10. Renewal of test solutions	Daily
11. Age of test organisms	7 days old
12. No. of organisms per test chamber	Five (5)
13. No. of rep. chambers per concentration	Eight (8)
14. No. of organisms per concentration	Forty (40)
15. Feeding regime	<i>Artemia</i> nauplii, minimum of twice daily
16. Test chamber cleaning	Immediately before test solution renewal
17. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
18. Dilution water	Filtered (1 μ m) seawater or Type 1 water salted up with Crystal Sea bioassay-grade salts.
19. Test concentrations	Ballast Water sample: 6.25%, 12.5%, 25%, 50%, and 100%, a Lab Water Control, and a ballast water control (from ballast water control tank).
21. Test endpoint	% survival and growth
22. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours of sample collection.
23. Sample volume required	3.1 liters per day
24. Test acceptability	80% or greater average survival in controls; average weight of control animals \geq 0.2 mg.

**SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR
INLAND SILVERSIDE, *Menidia beryllina*, LARVAL SURVIVAL AND GROWTH TEST OF
BALLAST WATER DISCHARGE (EPA-821-R-02-014, TEST METHOD 1006.0)**

1. Test type	Static renewal
2. Test duration	7 days
3. Salinity	5 to 32 ppt \pm 2 ppt
4. Temperature	25 \pm 1°C
5. Light quality	Ambient laboratory illumination
6. Light intensity	50-100 ft-c (10-20 μ E/m ² /s)
7. Photoperiod	16 hours light: 8 hours darkness
8. Test chamber size	600-mL to 1-L
9. Test solution volume	500 - 750 mL (according to loading & DO restrictions)
10. Renewal of test solutions	Daily
11. Age of test organisms	7-11 days old (\leq 24-h range in age)
12. No. of organisms per test chamber	Ten (10)
13. No. of rep. chambers per concentration	Four (4)
14. No. of organisms per concentration	Forty (40)
15. Feeding regime	<i>Artemia</i> nauplii twice daily
16. Test chamber cleaning	Siphon daily, immediately before test solution renewal
17. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
18. Dilution water	Filtered (1 μ m) seawater or Type 1 water salted up with Crystal Sea bioassay-grade salts.
19. Test concentration	Ballast Water sample: 6.25%, 12.5%, 25%, 50%, and 100%, a Lab Water Control, and a ballast water control (from ballast water control tank).
21. Test endpoint	% survival and growth
22. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours of sample collection.
23. Sample volume required	6 Liters per day
24. Test acceptability	80% or greater average survival in controls; average weight of control fish \geq 0.5mg

Test Quality Assurance Plan – Project Charlie
Volume III: Quality Assurance Project Plan (Shipboard
Testing)

Golden Bear Facility
Vallejo, California

Dept. 76408, Fund 46408
9 April 2012, Rev. A



Contents

Section 1	Summary	1
Section 2	Project Roles and Contact Information	2
Section 3	Data and Information	3
3.1	Facility Nomenclature Breakdown.....	3
3.2	Data Control	3
	Roles and Responsibilities	3
	Automation System and Online Information System	3
	BWTS Data Collection	4
	Documentation Verification	4
	Test Cycle Automation Initiation, Tracking, and Error Logging	4
	Red Lining of Procedures	5
	Hand Log Tracking and Filing	5
	Online Log Tracking and Filing	5
	Verification Data Record	6
	Report Data Verification.....	6
3.3	Data Quality Indicators.....	7
	Engineering Data	7
	Water Quality	9
	Biological Analysis.....	10
3.4	Data Reporting and Data Reduction.....	12
Section 4	Facility Preparation	13
4.1	Challenge Water Verification.....	13
	Continuous Data	13
	Grab Samples.....	13
4.2	Tank Cleaning and Inspection	13
	Tank Cleaning.....	13
	Final Inspection	14
Section 5	BWTS Commissioning	15
5.1	Arrival Inspection.....	15
5.2	Installation	15
5.3	Service	16
5.4	Operational Check.....	16
5.5	BWTS Shakedown Test	16

5.6	Lay-Up.....	17
Section 6 Biological Efficacy Test Procedures		18
6.1	Test Day Roles	18
6.2	Test Validation Parameters.....	19
6.3	Treatment System Operation	20
	Standby	20
	Ballasting Operation	21
	Back Flushing Cycle.....	21
	De-Ballasting Operations.....	21
6.4	Pumping and Piping Operation	21
	Flushing and Draining Prior to Testing	21
	Treatment Uptake	22
	Control Tank Uptake	23
	Flushing and Draining Between Fill and Discharge	23
	Treatment Tank Recirculation and Discharge	23
	Control Tank Recirculation and Discharge	24
6.5	Sampling Procedures	24
	Sampling Overview	25
	Sampling Coordination.....	26
6.6	Water Quality Analysis	26
	Continuous Data	26
	In-Situ Probe Measurements.....	27
	Grab Samples.....	27
6.7	Biological Efficacy Analysis	28
	Organisms Larger than 50 µm	29
	Organisms Larger than 10 µm, but Smaller than 50 µm – Required Assays	30
	Organisms Larger than 10 µm, but Smaller than 50 µm – Corroborative Assays.....	31
	Organisms Less than 10 µm	32
Section 7 Assessments		34

Appendix A: Project Training Requirements

References

- 001 *Environmental, Health, & Safety Plan*, Golden Bear Facility, October 2010.
- 002 *Quality Management Plan*, Golden Bear Facility, December 2011.
- 003 *Physical Plant Description*, Golden Bear Facility, October 2010.
- 004 *Project Plan for Project Charlie*, Golden Bear Facility, March 2012.
- 005 *Quality Assurance Project Plan (Land-Based Testing) – Project Charlie*, Golden Bear Facility, March 2012.
- 006 *Standard Operating Procedures – Project Charlie*, Golden Bear Facility, March 2012.
- 007 *Protocol for the Verification of Ballast Water Treatment Technologies*, US Environmental Protection Agency, September 2010.
- 008 *Guidelines for Approval of Ballast Water Management Systems (G8)*, Annex 4, MEPC 174(58), Marine Environment Protection Committee, 10 October 2008.

Section 1 Summary

The Quality Assurance Project Plan (QAPP) establishes the quality assurance/quality control measures for executing the SOPs, the operating parameters and data collection requirements, and the protocols for evaluating test biological and chemical conditions. This QAPP is specific to the evaluation of the Project Charlie ballast water treatment system (BWTS) for Shipboard testing in accordance with the International Maritime Organization (IMO) *Guidelines for Approval of Ballast Water Management Systems (G8)* and the US EPA Environmental Technology Verification (ETV) protocol to the extent reasonable.

The Shipboard QAPP is one part of the Test Quality Assurance Plan (TQAP) that also consists of Project Charlie specific Project Plan (Plan) and Standard Operating Procedures (SOPs). This QAPP also references the Facility Quality Management Plan (QMP). Wherever a conflict exists between documents, the SOPs takes first precedence, and the QAPP takes second precedence.



Photo 1. Facility Operations

Section 2 Project Roles and Contact Information

Project roles and responsibilities are as described in the Program Documentation *Quality Management Plan*. The key personnel contact information for this BWTS project is as follows:

William T. Davidson, Facility Director/Chief Engineer

Golden Bear Facility
707.654.1304, cell 707.592.4267
bdavidson@csum.edu

Richard S. Muller, Facility Manager/Quality Officer

Golden Bear Facility
707.654.1258
rmuller@csum.edu

Veronica Boe, Facilities Program Administrator

California Maritime Academy
707.654.1156
vboe@csum.edu

Nicholas A. Welschmeyer, PhD, Lead Investigator

Golden Bear Facility
831.771.4439
welschmeyer@mlml.calstate.edu

Harry Bolton, Ship Master

California Maritime Academy
707.654.1192, cell 707.246.7495
HBolton@csum.edu

Jad Mouawad, Verification Organization

Det Norske Veritas
+47 480 50 902
Jad.mouawad@dnv.com

Andrew Daley, BWTS Project Manager

Trojan Technologies
519.457.3400, cell 226.688.7430
adaley@trojanuv.com

ES&H Contacts—Moss Landing Marine Laboratories (MLML), 831.771.4400
Jocelyn Douglas, MLML Health and Safety Officer, 831.771.4451, cell 831.750.9563

Section 3 Data and Information

This section outlines the procedures involved in the control of, quality indicators for, and reduction validation of project data and information.

3.1 Facility Nomenclature Breakdown

The facility uses specific nomenclature to describe various activities. The following provides definitions, and applies this nomenclature to Project Charlie.

Project. A Project is a collection of tests to verify a technology claim, or collection of experiments to prove a thesis. Project Charlie consists of four Shipboard tests to verify that the BWTS meets the IMO D-2 standard. The project will be performed in accordance to the IMO G8 Guidelines and where possible the guidance of the ETV protocol.

Test. A Test is one replicate activity that compares the performance of a treatment system (or other method) to a control. Each Project Charlie Test consists of a parallel treatment uptake through the treatment system and control uptake with no treatment. These two separate ballast water parcels have similar challenge conditions as they are ballasted simultaneously. The two parcels are held separately in a “treatment” tank and a “control” tank. Discharge is sequential. The treatment is discharged through the treatment system after a designated hold time. The control is discharged without treatment after a similar hold time.

Event. The Facility uses the term Event to describe a discrete combination of activities, typically performed without stopping, resetting, or other breaks. It typically takes several Events to complete a Test.

3.2 Data Control

Each project related task is tracked either through the automation system, online information system, or hand logs which are later scanned into the online information system.

Roles and Responsibilities

The Quality Officer (Quality) reports directly to the Facility Director. During shipboard trials Quality operates under the supervision of the Chief Engineer, and is responsible for the data and information procedures outlined in this section, and detailed in the various Standard Operating Procedures (SOPs). The Lead Operator (Operator) is designated by the Chief Engineer, and is responsible for all pumping and piping operations. The Lead Scientist (Scientist) is responsible for all sampling and analysis operations. The Operator and the Scientist, while they do not report to Quality, are responsible to provide Quality with the data and information outlined in this section. Facility personnel roles are further explained in the Facility Environmental, Safety & Health Plan, and the Facility Quality Management Plan.

Automation System and Online Information System

The automation system is outlined in the Facility Physical Plant Description. The automation system is comprised of a computer network which monitors and securely records a wide array of field sensors such as valve position indicators, sample flow meters, and water quality instruments. User interfaces are located through-out the Facility to provide visual indicators

pipings system, BWTS, and sampling system status to the Facility team. Further, the automation system facilitates the generation of reports based on the stored data.

The online information system is a computer network for handling Facility documentation. Access is enabled for personnel to view standard and test specific procedures, access secure collected data, and manipulate data into useful information for reports. User forms allow online entry of data directly into the information system. Hand logs are scanned into the system.

BWTS Data Collection

The BWTS is expected to have its own data collection system in accordance with the IMO G8 Guidelines. This data shall be downloaded and transferred to the Facility information system not less than once per test.

Documentation Verification

In consultation with the Director, Science, and Operations, Quality will confirm the correct versions of the Facility standard documents, and project specific documents. Quality will track this in the datasheet, and confirm the correct versions are available online.

Quality, prior to the start of a test cycle, will determine which aspects of the automation system will be used for data monitoring, and where the Facility team will need to maintain hand logs. To make this assessment, Quality will open, view, practice, and determine the availability of each of the data collecting tools in the automation system.

Upon this determination, Quality will update the Quality Data Sheet to select which data collection will be by Hand Log and which will be Online.

Quality will distribute the documentation list to all team members.

Test Cycle Automation Initiation, Tracking, and Error Logging

A test cycle is one complete ballasting evolution including: uptake to Treatment and Control, a holding period, and finally discharge of the treated and control ballast water.

Before start of a test cycle, the Operator and Scientist will provide an automation and online system status report to Quality. The report will include: calibration report for field instrumentation; system check of all monitored parameters; system check of all screens, forms, and reports. An action plan for hand monitoring and logging of non-functional items will be submitted. Quality will scan the report into the online information system.

At the start of a test cycle, before any data logging or pumping activities, Quality will initiate the automation to begin data logging. The automation system will automatically generate a unique test event number and begin logging of all monitored parameters. This unique number will be matched to all monitored data and all online data sheets in the online system. The automatically generated test event number will be based on the date and time of the start of the effort. For example, if initiation occurs at 1:32 pm on March 14th, 2012, the test number would be: "12-03-14-1332."

Before the test event, Quality will perform the automation system checks as identified in the data log. In addition, Quality will communicate with the Operator and Scientist to identify any automation system errors. Automation errors found independently, or highlighted by

team members, will be documented and made part of the data record. Where possible, hand records of that monitored parameter will replace the automated entry.

At the conclusion of the test event, the Quality Officer will gain confirmation from the Lead Operator and stop the automatic collection of data.

Red Lining of Procedures

As testing efforts progress, the complexity of the operations and variability of the equipment and challenge water conditions, it may at times be advantageous or imperative to change procedures.

For example, the treatment system following a back flush cycle fails to open its valve. In this case, the Operator may use ordinary means, such as manually opening the valve, to allow the test to continue.

For example, a sampling net fails allowing some part of its contents to spill into the sample tub. In this case, the Scientist might continue sampling to that net, but analyze its contents separately from the other two nets, and note average results until it is determined that all results are consistent.

In any case, where there is a deviation from the test procedures, protocol, plan, etc. the person-in-charge of that operation must “red-line” that document. Redlining is accomplished by:

- Strike a single line, using indelible ink, through the affected text, figure, chart, or other item.
- Initial each adjacent to each and every strike.
- Provide, using indelible ink, any required correction in the form of text, figure, chart, or other item.
- Provide an explanation in the “notes” section of why the change was made.
- Date and sign each affected page.

Hand Log Tracking and Filing

At the end of each test cycle, the Quality Officer will manually collect all hand logs from team members, and scan these into the online information system. Each hand log will be assigned the test number and an identifier associated with that log. For example, the BE sampling log for the above test event would be: “2012-03-14-1332-BESAMPLING.”

Daily during the test cycle, the Quality Officer will view automated reports and online data logs to confirm that the systems are working correctly.

The Quality Officer will then continue to collect additional hand logs as they are completed, scanning these into the online information system.

Online Log Tracking and Filing

Daily during the test cycle, the Quality Officer will review and track all online logs using the Quality Data Sheet. A copy of utilized logs will be copied to a secure location in the information system, and named using the same convention as for the hand logs.

Verification Data Record

Following each test cycle, the Quality Officer will make one (1) copy of the automation system database, the scans of hand data, and the online data forms to a secure read only DVD. At the end of a Test two (2) copies will be made. Each DVD will be scribed with test name, date, and copy recipient. For example, a DVD for test day March 16th, would read: “Test 12-03-14-1332, Data Recorded 12-03-16, Operator Copy.” The DVD copy is kept on-site by Quality. Upon return, Quality is to provide a documentation package to the Facility as per the Quality Management Plan.

Report Data Verification

Quality will review all Facility reports for conformance with the Data Verification Records. Discrepancies will be documented and provided to the Director.

3.3 Data Quality Indicators

Statistical analyses will be carried out on data obtained for all performance measurements. As part of the assessment of data quality, six data quality indicators (DQIs) will be used to interpret the degree of acceptability or utility of the data. Data quality will be reported in a table comparing these objectives and criteria, to recorded results. The following protocol will be used to assess the following DQIs, as well as acceptable limits and criteria for:

- Representativeness
- Accuracy
- Precision
- Bias
- Comparability
- Completeness

At the conclusion of each test, the data will be processed and compared to the below assigned DQI targets. These are not Pass/Fail targets, but rather an indicator of the quality of the collected data. Where data does not meet its target, an explanation is required. In subsequent tests, either the procedure will be modified to improve data quality, or the target will be lowered.

Engineering Data

Engineering data is collected and stored by the automation system for post processing. In general, the data follows expected trends through each test event.

For example, recorded tank levels will follow the trend of the recorded pumping rate. A rate of 250 cubic meters per hour should result in a level corresponding to 250 cubic meters of ballast water in the tank after one hour.

For example, recorded temperatures should agree with the water quality monitoring system and there should be general agreement between the various sensors in the system. If the temperature monitor that is recording at the pump discharge reads 20 Celsius, and the temperature monitor at the treatment system discharge reads 10 Celsius this does not follow the expected trend, and needs to be investigated.

The instrumentation is calibrated by a combination of factory set points, and practical tests. For example, the sample tubs have graduated marks against which the sample flow meters total volume counters can be compared.

Table 1 - Data Quality Indicators for Engineering Parameters

Parameter	Indicator	Metric	Description
Ballast Water Flow - Rosemount 8711 Flow Meter	Representativeness	90%	Flow meters are continuously online and recording. A temporary system fault is possible.
	Accuracy	+/-3%	Instrument performance is +/-0.3% of flow as per factory calibration.
	Precision	0.1	Cubic meters per hour. Display on unit.
	Bias	NA	
	Comparability	10%	All ballast water passes at least two of the three flow meters, allowing a comparison between them.
	Completeness	90%	Data is automatically recorded in the automation system.
Ballast Water Volume - TMS LevelCom Tank Level Indicator	Representativeness	90%	Indicator measures the water column in the forward portion of the tank. If ship is trimmed aft, it will miss a small portion of this water when the tank level is very low.
	Accuracy	+/-5%	Some variation is expected due to the shape of the tanks changing at various heights.
	Precision	0.1	Meters. This is a function of the system analog signal to the automation system.
	Bias	NA	
	Comparability	10%	Variation when compared to flow meter and time calculation while tank is being filled/emptied.
	Completeness	90%	Data is automatically recorded in the automation system.
Sample Water Flow - Seametrics SPX	Representativeness	90%	Flow meters are continuously online and recording. A temporary system fault is possible.
	Accuracy	+/-5%	Instrument performance is +/-1% of full scale.
	Precision	0.3	Gallons per minute on display unit.
	Bias	NA	
	Comparability	10%	Variation between the three sample tubs that take simultaneous samples.
	Completeness	90%	Data is automatically recorded in the automation system.

Water Quality

Water quality measurements will be conducted with a combination of field installed continuous monitoring equipment, and discrete sampling conducted by either hand held in-situ instrumentation or laboratory analysis.

The continuous monitoring provides real time viewing and verification, as well as a continuously recorded set of readings.

Table 2a – Continuous Monitoring Parameters

Sensor	Maker	Type	Calibration Procedure	Reporting Units	Range	Accur-acy	Preci-sion
Temperature	Sea-Bird Electronics	SBE 21	Nominally 1/year	degrees C	-5 to +35	0.01	0.001
Conductivity	Sea-Bird Electronics	SBE 21	Nominally 1/year	S/m	0 to 7	0.001	0.0001
Transmiss-ometer (25 cm)	Wet Labs	C-Star	Nominally 1/year	% transmittance or beam attenuation	0-100	N/A	N/A
Dissolved Oxygen	Sea-Bird Electronics	SBE 43	Nominally 2/year	mg/L	120% of saturation	N/A	N/A
Fluorometer	Turner Designs	Cyclops-7	In-house, before each experimental run	ug/L	0.1 to 50	+/- 5%*	0.01

* When compared to wet chemistry.

Table 2b – Grab Sample Monitoring Parameters

Parameter	Maker	Type	Calibration Procedure	Reporting Units	Range	Accuracy	Preci-sion
Temperature	YSI	6560	Nominally 1/year	Degrees C	-5 to +50	0.15	0.1
Conductivity	YSI	6560	Nominally 1/year	S/m	0 to 100	0.5%	0.1
Dissolved Oxygen	YSI	6150	Nominally 1/year	mg/L	0 to 50	0 to 20: ± 0.1 or 1%, whichever is greater	0.01
Chlorophyll α	YSI	6025	In-house per exper. run	$\mu\text{g/L}$	0 to 400	0.1 $\mu\text{g/L}$ Chl	~ 0.1
Turbidity	YSI	6136	In-house per exper. Run	NTU	0 to 1000	± 0.1	0.1 NTU
pH	Beckman	70	In-house per exper. Run	pH units	0 to 14	± 0.1	± 0.03
Total Suspended Solids (TSS)	Proweight	Filter	Calibrated 1/year, checked per exper. run	mg/L	5 to 100	± 2	2.60%
Particulate Organic Carbon (POC)	GF/F	Filter	In-house per exper. Run	mg/L	0.1 to 100	± 0.15	7.50%
Dissolved Organic Carbon (DOC)	GF/F	Filter	Per exper. run, at McCampbell Analytical, Inc.	mg/L	0.5 to 100	± 0.1	20%

Biological Analysis

Data quality indicators (DQIs) are determined empirically by the GBF science staff based on actual sample measurements made in the GBF laboratory. By design, the Facility has developed procedures that generate a high level of replication.

Table 3 - Data Quality Indicators for Engineering Parameters

Sample Description	Analytical method	Data Quality Indicators (DQI)*	Notes
1. Organisms >50 µm (#/m ³)	Visual determination of live status (30x magnification)	Expected CV** <31%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
2a. Organisms 10–50 µm Method A MPN (MPN/mL)	MPN determination of cultivable phytoplankton; fluorometric determination of growth	Expected CV <60%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
2b. Organisms 10–50 µm Method B Flow cytometry (#/mL)	Flow cytometric determination of live cells tagged w/ fluorescein diacetate (FDA)	Expected CV <42%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
2c. Organisms 10–50 µm Method C Epifluorescence microscopy (#/mL)	Visual epifluorescence determination of live cells tagged w/ FDC and CMFDA	Expected CV <25%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities (experience limited to one complete ballast sequence)
3a. Organisms < 10 µm Bulk bacterial plate counts (CFU/mL).	Total colony forming units (CFU) on agar substrate	Expected CV <90%.	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
3b. Pathogens <i>E. coli</i> (MPN/100 mL)	MPN determination IDEXX proprietary, enzyme-based MPN kit (Colilert™)	Expected CV <64%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
3c. Pathogens <i>Enterococci</i> (MPN/100 mL)	MPN determination IDEXX proprietary, enzyme-based MPN kit (Enterolert™)	Expected CV <42%	DQI based on 'uptake' and 'control' samples which provide highest live organism densities
3d. Pathogens <i>Vibrio cholerae</i> serotype 01 (detection limit <1CFU/mL)	Antibody detection; New Horizons Diagnostics, Cholera Smart™ II	N.A.	No observation of <i>V. cholera</i> (Type 01) >1 CFU/100 mL has been found in any sample analyzed at GBF (n=36)
3e. Pathogens <i>Vibrio cholerae</i> serotype 0139 (detection limit <1CFU/mL)	Antibody detection; New Horizons Diagnostics, Bengal Smart™ II	N.A.	No observation of <i>V. cholera</i> (Type 0139) >1 CFU/100 mL has been found in any sample analyzed at GBF (n=36)

* Data quality indicator (DQI) is estimated as twice the empirically measured coefficient of variation (CV) for sample replicates analyzed by GBF science staff for the given assay.

**CV = Standard Deviation/mean*100, where the sample standard deviation is

$$\sqrt{\sum(x - \bar{x})^2 / (n - 1)}$$

3.4 Data Reporting and Data Reduction

All test data for BWTS testing will be collected, analyzed, and reported in a uniform format. Specifically, all data from time-integrated sample collections; e.g., net samples and time-integrated microbe/chemistry ‘grab’ samples, will be collected in triplicate, corresponding to the three individual sampling ports configured at the biological sampling station.

Additionally, each replicate sampling container, e.g., each net and each 20 L ‘grab’ carboy, will be sampled in triplicate, again, for sample aliquots corresponding to the assays described in the Science SOPs.

Sample means and sample standard deviations will be calculated and reported as figures and tables as shown in the example dataset below (the number of replicates will be indicated). Note that the example below represents data reporting formats for the same raw data, e.g., the figure is a graphical representation of the tabular data. In effect, each ballasting sequence (uptake, control, treatment) will yield 3x3=9 analyses of the stated scientific measuring parameters. Example formats for data reporting are provided below.

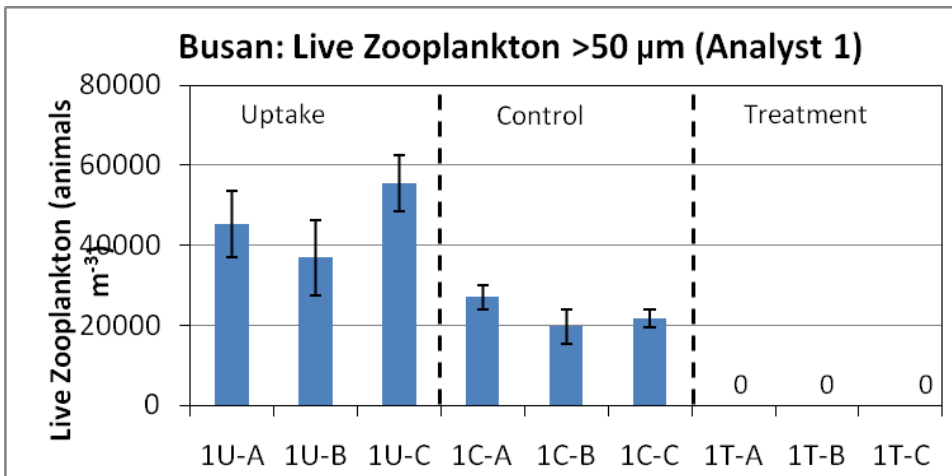


Figure 1 - Data Reduction Figure Example

Table 4 - Data Reduction Table Example

Location	Sample	Live Zoop. (#/m³)		Dead Zoop. (#/m³)		% Live	
		Mean	S. Dev.	Mean	S. Dev.	%	n
Busan (Uptake)	1U-A	45425	8282	23523	3351	65.7	3
	1U-B	36907	9328	17643	3219	67.4	3
	1U-C	55564	6965	18048	3337	75	3
Busan (Control)	1C-A	27004	3096	16956	5042	61.9	3
	1C-B	19684	4219	12845	2128	60.3	3
	1C-C	21750	2110	11136	1437	66	3
Busan (Treatment)	1T-A	0	0	4857	1052	0	3
	1T-B	0	0	3879	433	0	3
	1T-C	0	0	6649	823	0	3

Section 4 Facility Preparation

This section provides specific activities that the Facility will conduct in preparation for initial testing and efforts between testing cycles.

4.1 Challenge Water Verification

Continuous Data

Natural water for the BWTS will be brought on at various California coastal and river areas during *T/S Golden Bear*'s 2012 summer training cruise. All testing will be performed with ambient water, without adding or concentrating of any chemical or biological components.

GBF operates an inline Sea Bird SBE 21 thermosalinograph (TSG) during shipboard ballast testing. The data, including temperature, conductivity, salinity, dissolved oxygen, transmissivity (WetLabs Transmissometer) and chlorophyll fluorescence (Turner Cyclops), is displayed for the user in real-time on a computer screen and recorded to disk. The data is post processed according to Sea Bird's recommendations.

Grab Samples

Grab samples of the challenge water during testing is described later in the *Biological Efficacy Testing* section of the QAPP.

4.2 Tank Cleaning and Inspection

Following guidance in the data sheets provided in the SOPs, the GBF Lead Operator and Quality Officer will ensure proper tank conditions are achieved prior to commencing testing. Testing protocols require that the Treatment Tank (3-154-1) and Control Tank (3-154-2) be opened, cleaned as required, inspected, and approved by the Lead Operator as "ready for testing" before Shipboard testing commences. The GBF Lead Operator will contract with a tank cleaning contractor with all proper certifications and coverage for work aboard the vessel.

Tank cleaning is not necessary between subsequent Shipboard tests.

Tank Cleaning

The tanks will be opened at manhole access covers for each tank (2 each), the covers shall have safety rails installed, and mechanical portable ventilators shall be installed at each tank's weather deck vent terminus. The tanks shall be ventilated continuously throughout the entire tank cleaning process. After no less than 12 hours of ventilation, a certified marine chemist will test the tanks as "safe for men" and tank cleaning can commence. The tanks will be checked by a certified competent person for continued safe condition at the beginning and end of each work day. All tank entry documentation shall be maintained for inspection at the tank entry point.

An initial inspection by the Lead Operator and contractor after the first rinse will determine how much, if any, mucking and disposal of silt or debris is required. The silt will be mucked

and loaded to appropriate containers staged on deck for later disposal as per local requirements.

Cleaners outfitted with appropriate Personal Protective Equipment (PPE) will use high pressure washers to spray down the Control Tank surfaces with water, and the Treatment Tank surfaces with a 200 ppm chlorine solution. After a contact time of not less than five minutes, all surfaces will be rinsed with city fresh water using the same high pressure washers. The wash down from the cleaning will be continuously pumped out of the ballast tank and transferred to municipal waste.

A final inspection by the Lead Operator and contractor will confirm the removal of silt, debris, and wash water. In addition, random sampling of six (6) wet tank surface locations in the Treatment Tank will be conducted. A reading of less than 3 ppm free chlorine is considered passing.

Final Inspection

The tanks will be pumped “dry,” with no standing water, and left ventilating for an additional 24 hours. Final inspection will be performed by the GBF Lead Operator, Quality Officer, or assignee for acceptance, at which time the tank will be closed up and ventilation removed. The tanks will be offered for inspection to the BWTS representative and the Verification Organization prior to closing.

Section 5 BWTS Commissioning

This section provides specific procedures required for commissioning the BWTS. A successful commissioning will integrate the system with the Facility, and confirm proper mechanical operation in accordance with the BWTS instructions.

All commissioning efforts are performed by the Lead Operator (Operator). Checklists and other documentation are provided to the Quality Officer (Quality) on a daily basis. Commissioning data sheets are provided in the Standard Operating Procedures (SOPs).

5.1 Arrival Inspection

Upon arrival of the BWTS at the facility staging area, an initial inspection will be performed to determine delivery condition and readiness for install. Utilizing the “BWTS Commissioning Data Sheets,” the GBF Lead Operator or assignee, the rigging and installation contractor, and Quality will inspect the system for:

- Overall condition and damage indication.
- Condition of the ISO container rack.
- Ballast water inlet and outlet connections (8-inch flange).
- Drain connection (4-inch flange).
- Electrical load requirements.
- Cable lengths and sizes for GBF-provided connectors.
- Fresh water and compressed air requirements.
- Any additional requirements or conditions requiring specific care or address.

5.2 Installation

Utilizing the “BWTS Commissioning Data Sheets,” the GBF Lead Operator or assignee will ensure that the BWTS is properly installed, secured, and that all mechanical, electrical, and plumbing connections are made up.

Based on standard requirements for installing a 20-foot ISO container on the designated location onboard the *TS Golden Bear* and any unique requirements identified at the arrival inspection, the GBF Director will utilize a sub-contract to:

- Provide crane, rigging, and mechanical service to shift and secure the BWTS to the vessel
- Install all plumbing interfaces, including ballast in and out, drain, air, and water, along with any unique interfaces such as chemical injection or sampling lines.

Crane and rigging services will be planned and scheduled with facility and vessel personnel at least 24 hours prior to commencement, to allow for notification and alternate arrangements for planned events. A suitable mobile crane and operator will be employed, along with a rigging crew to shift the BWTS from the CMA waterfront parking lot to the end of the pier near the stern of the vessel. The interference radius of the vessel’s aft crane shall be marked

out, and suitable blocking placed to allow the mobile crane to lift and set the ISO container onto the 01 Aft Deck of the vessel just outside this turning radius. The contracted rigging crew and crane operator will then employ the vessel's crane to shift the container into position and correct alignment for placement and securing to the container platform. The vessel's Chief Mate will observe, inspect, and approve the securing of the container.

Electrical power interfaces will typically be installed by qualified facility personnel under direction of Chief Engineer, though an electrical contractor with facility oversight may be employed. Depending on the complexity of the BWTS user interface, the inter-connection to the Integrated Monitoring and Control system (IMACS) will be performed by qualified facility personnel or contracted to the vessel's automation contractor.

5.3 Service

Utilizing guidance from the BWTS Technical Bulletin and the "BWTS Commissioning Data Sheets," the GBF Lead Operator or assignee will ensure that the system will be inspected and serviced by facility personnel and/or an OEM Technical Representative under facility supervision prior to placing BWTS in operation. In general, this service would include inspection of UV lamps, filter installations, freedom of mechanical linkages and pump shafts, operation of fitted valves and handwheels, and control interface condition. Any installed lubrication points shall be checked or lubricated as per BWTS Technical Bulletin.

5.4 Operational Check

Utilizing guidance from BWTS Technical Bulletin and the "BWTS Commissioning Data Sheets," the GBF Director or assignee will initiate operation of the BWTS. The system will be placed into operation readiness by checking all system elements for proper installation and gradually bringing them on line, which will include the following checks:

- The booster pump will be checked for proper rotation.
- Piping connections and covers will be checked under system pressure.
- Flow rates will be determined and confirmed.
- Electrical power and controls integration will be initiated and confirmed.

5.5 BWTS Shakedown Test

Shakedown subjects the BWTS to stressing conditions that are within the BWTS's specified limits. The purpose is to ensure that the BWTS will not have mechanical failures during BE Testing. The shakedown steps are detailed in the "BWTS Shakedown Test Data Sheets" located in the SOPs, and are outlined below:

- Sea-to-Sea for two (2) hours at ~250 m³/hr treatment.
- Starts and stops of facility pump and BWTS.
- Overnight idle period.
- Sea-to-Sea for four (4) hours at ~275 m³/hr treatment (110% of capacity).
- Uptake cycle operational test to determine expected conditions
- Two (2) hour uptake sampling test to determine expected plankton net conditions

5.6 Lay-Up

After commissioning and shakedown test, and between test cycles, the system will be laid up as per the “BWTS Commissioning Data Sheets.” The GBF Director or assignee shall oversee the performance of these lay-up procedures based on Technical Bulletin recommendations and guidance. In general, these procedures will consist of flushing the system with fresh water and opening power supply breakers to the BWTS, thus leaving in “wet” lay-up while installed on the vessel. For idle periods in excess of 1 month or if extended freezing periods are expected, the lay-up will also consist of draining the unit and filter on the BWTS.

Section 6 Biological Efficacy Test Procedures

This section provides the procedures for the biological efficacy testing. This includes ballast water uptake and discharge procedures, sampling collection and analysis procedures. The section is divided into the following parts:

- Test day roles.
- Test validation parameters.
- Treatment system operation.
- Pumping and piping operation.
- Sampling procedures.
- Water quality analysis.
- Biological efficacy analysis.

6.1 Test Day Roles

The conduct of a biological efficacy test requires careful coordination of pumping and piping systems, treatment system operations, sample collection, sample analysis, water quality continuous monitoring, water quality grab samples, and most importantly quality control. To facilitate these operations, the following identifies the roles of key personnel related to completing these tasks.

Given the small size of the Facility, persons will play multiple roles. As such, the below description is listed by person.

Chief Engineer Bill Davidson.

- Test Conductor. Ensures that treatment system operation, pumping and piping operations, and sampling collection activities are coordinated and properly timed.
- Equipment Responsible Party. Responsible for all mechanical equipment involved with pumping and piping.
- Operator Supervisor. Reviews testing plan with Operator such that the pumps and piping system are properly operated.

Rich Muller.

- Quality Officer. Ensures that the procedures being utilized are current, and that all documentation is completed and logged into the automation system. Starts and stops the event. Checks function of the automation system during a test.
- Water Quality – Continuous Monitoring. Ensures that the continuous water quality system is calibrated, operating, and properly logging data. Provides feedback on water quality to the Scientist during decision making time before starting an uptake.
- Facility Manager. Ensures that all equipment and laboratory facilities are set-up for test day. Ensures that facilities are properly cared for, and secured following use.

Nicholas Welschmeyer.

- Principal Investigator – Scientist. Ensures that the procedures are conducted in accordance with scientific protocols. Responsible for validating test parameters, and conducting validity discussions with VO.

- Sampling Team Lead. Ensures that sampling system is properly operated, and that required volumes are collected and processed.
- Biological Analysis. Ensures that biology analysis team conducts assessments in accordance with protocols. Reviews and assists when problems and questions arise. Reviews preliminary data, conferring with VO if and as needed.
- Water Quality – Grab Samples. Ensures that discrete, grab, samples are obtained, handled, and sent to the appropriate laboratories (internal or external). Receives and analyzes reports.

Staff with Single Task Assignments.

- John Coyle. Lead Operator and Relief Chief Engineer of ship.
- Dan Lintz. Ballast System Operator and Chief Mate of ship.
- Brian Maurer. Phytoplankton Analysis and Facility Laboratory Lead.
- Jules Kuo. Zooplankton Analysis Lead.

6.2 Test Validation Parameters

The test validation parameters are provided in the Project Plan. These are duplicated here for convenience. Please refer to the Project Plan for a more detailed explanation of these parameters, actions to perform if one or more parameters are not met, and interpretation of levels and durations. The remainder of this section will list the baseline numbers without error bands or qualifiers for simplicity. However, the actual criteria is as listed in the Project Plan Valid Test Parameter Table.

Table 5 – Valid Test Parameters, One Shipboard Test

	Criteria	Uptake Treatment	Cycles Control	Discharge Treatment	Cycles Control
Treatment Line and Tank					
Average (m3/hr)	250 ± 10%				
Total Volume (m ³)	≥ 200				
Control Line and Tank					
Average (m3/hr)	250 ± 10%				
Total Volume (m ³)	≥ 200				
Combined Sample Volume (m³)					
Uptake and Control Discharge	≥ 3				
Treatment Discharge	≥ 9				
Ballast Hold Duration (hours)	48+				
Water Quality					
Salinity (PSU)	N/A				
Temperature (Celcius)	N/A				
DOC (mg/L)	N/A				
POC (mg/L)	N/A				
TSS (mg/L)	N/A				
Uptake Living Populations					
≥50 microns (organisms/m ³)	100				
≥10 < 50 microns (organisms/mL)	100				
Control Living Populations					
≥50 microns (organisms/m ³)	10				
≥10 < 50 microns (organisms/mL)	10				

, but may be utilized
if indicated.

6.3 Treatment System Operation

The BWTS includes a filtration system and a UV device. The filtration device is bypassed during de-ballasting. The BWTS is controlled locally or remotely by the Facility automation system. The automation system can place the BWTS in the following modes: Standby, Ballast, and De-Ballast. In addition, the BWTS control system provides the Facility automation system with alarm and fault status signals.

Standby

All operations start with the BWTS in Standby. The Standby mode is engaged by the “stop” command from the Facility automation system. This means that power is supplied to the BWTS allowing operation of the control system, electrical enclosure heaters, etc. All valves in the BWTS are automatically closed.

The BWTS can enter Standby from the Ballasting mode. In this case, the Facility selects Standby (“Stop” on the Facility user interface) and the BWTS automatically starts a backflush

cycle, then enters a UV lamp cool-down period, and then closes all BWTS valves. Entering Standby from Deballasting is similar, but without the back flush step. The Facility will then close valves to isolate the BWTS.

Ballasting Operation

For a ballasting operation, the Facility will prime the BWTS inlet piping and bleed air at the BWTS inlet. The Facility will then place the BWTS into Ballast mode.

The BWTS then automatically undergoes its start-up procedure: opens the filter inlet valve, ensure the filter bypass valve is closed, and turns on the UV lamps. Once the UV lamps have reached operational output temperature, the BWTS will automatically open the outlet valve and allow fully treated water to pass through to the ballast tanks.

Back Flushing Cycle

At certain intervals, based on differential pressure, the BWTS will initiate a back flush cycle. In this timed cycle a percentage of inlet ballast water is used to back flush the filter elements with the effluent sent overboard to waste. The system is designed to deliver treated effluent at a constant rate, however delivery may be reduced during these back flush periods which could slow uptake. Ballast system pumping and sampling operations do not change during these periods.

De-Ballasting Operations

For a de-ballasting operation, the Facility will prime the BWTS inlet piping and bleed air at the BWTS inlet. The Facility will then place the BWTS into De-Ballast mode.

The BWTS then automatically undergoes its start-up procedure: ensures the filter inlet valve is closed, opens the filter bypass valve, and turns on the UV lamps. Once the UV lamps have reached operational output temperature, the BWTS will automatically open the outlet valve and allow fully treated water to pass through to the ballast tanks.

6.4 Pumping and Piping Operation

Tests are the ballasting uptake and discharge operations that specifically utilize the Treatment Tank and Control Tank, and include efficacy testing to compare uptake and discharge organism counts for the Treatment Tank and Control Tank.

The Shipboard “Piping System Line-up Diagrams,” and the “Shipboard Operation of BWTS and Facility Piping System” are located in the SOPs, which provide detailed instructions and logs for running the BWTS and Facility piping system. The following section outlines the testing procedures.

Flushing and Draining Prior to Testing

The piping system must be flushed with a mild bleach solution and then drained prior to a Shipboard test uptake.

Treatment Uptake

The objective of the uptake is to fill the Treatment Tank (3-154-1) through the BWTS at a rate of 250 m³/hr and until the Treatment Tank volume is not-less-than 200 cubic meters. Suction is taken from a seachest.

The actual steps of the treatment uptake are detailed in the SOPs. A high level review is provided here.

- Piping system is arranged to take suction from the sea with the treatment pump, and deliver water to the BWTS inlet. Until the BWTS is running, the water recirculates through the piping system and is returned to the pump suction.
- The treatment pump is started, and air is bled from the system.
- The BWTS is turned on and set to Ballast Mode.
- Piping system is managed for the pump to be at the target flow of 250 m³/hr.
- Once the BWTS is warmed up and online it will automatically open its outlet valve, allowing treated ballast water to flow through discharge piping to the overboard.
- The piping recirculation route is closed ensuring all water flows through the BWTS to the overboard.
- Flow rate is managed using the ballast treatment pump and piping system.
- Water Quality Monitors, providing continuous monitoring of the ballast water at the pump discharge, are placed online.
- The sampling system is flushed with raw seawater while suction continues to be taken from the sea and discharges to the overboard.
- Once the sampling system is flushed and ready to begin collection (at the same time):
 - The Treatment Tank (3-154-1) fill valves are opened, and the discharge to overboard is shut.
 - Sampling begins by placing the nets in front of the flowing sample water, and logging the start into the automation system.
- Flow rate management occurs as discussed above until the desired tank level is reached.
- Treatment Uptake is completed by (at the same time):
 - Treatment Tank is confirmed at a volume not-less-than 200 m³.
 - Sampling system is switched so that sampling begins in the next set of tubs.
 - Discharge from the BWTS is sent from the Treatment Tank to the Control Tank.
 - The Treatment Tank inlet piping is double blocked and bled.

Control Tank Uptake

Following Treatment uptake, the system is switched over to begin filling the Control Tank. The Control uptake continues to take water from the seachest. After the switch is complete, the BWTS is shutdown and isolated. The Control Tank is filled to a volume not-less-than 200 cubic meters.

- Once BWTS discharge is switched to send water to the Control Tank, the 01 Deck by-pass valves are opened, and the BWTS is shutdown.
- Flow management occurs as discussed above.
- Sampling for Treatment Uptake stops, and sampling for Control Uptake begins.
- Once the BWTS is completely shutdown, it is isolated from the system.
- Control uptake is completed by (at the same time):
 - Control Tank is confirmed at a volume not-less-than 200 m3.
 - The treatment pump is stopped.
 - All remaining valves left open are secured.


Flushing and Draining Between Fill and Discharge

The piping system must be flushed with a mild bleach solution and then drained between the filling and discharging cycles to avoid contamination.

Treatment Tank Recirculation and Discharge

Note: The Treatment Tank must be discharged BEFORE the Control Tank.

Following a holding time of not-less-than 48 hours, the Treatment Tank will be discharged overboard as detailed in the SOPs. The ballast water will be sampled in triplicate at the Main Deck sample port in accordance with the SOPs.

- The piping system is arranged to take suction from the Treatment Tank with the treatment pump, and deliver water to the BWTS inlet. Until the BWTS is running, the water recirculates directly to the pump suction.
- The treatment pump is started, and air is bled from the system.
- The BWTS is turned on and set to De-Ballast Mode. 

If filtration is desired, set to Ballast mode with filter backwash returned to holding tank.
- Flow management occurs as discussed above.
- Once the BWTS is warmed up and online it will automatically open its outlet valve, allowing treated ballast water to recirculate. The direct pump recirculation route is closed.
- Flow management occurs as discussed above.
- Water Quality Monitors are placed online.
- The sampling system is flushed with treated ballast water while the system continues to recirculate water from the Treatment Tank.
- Once the sampling system is flushed and ready to begin collection (at the same time):

- The discharge is sent to overboard and the treated water recirculation route is closed.
- Sampling begins by placing the nets in front of the flowing sample water, and logging the start into the automation system.
- Tank levels are monitored as follows:
 - Pumping rate of 250 m³/hr is maintained until tank water level reaches ~300 mm in height.
 - Pumping rate is decreased to 50 cubic meters per hour starting at 300 mm in height, until the pump loses suction.
- When the treatment pump loses suction, the BWTS and pump are secured.
 - The BWTS is shutdown.
 - The treatment pump is stopped and the piping to overboard is closed immediately. This ensures that the BWTS remains flooded during its shutdown cycle.
 - All remaining valves left open are secured.
- Sampling system flow will naturally stop, once the ballast pump loses suction and is no longer pumping ballast water. The sampling system inlet valves are then shut, and the sampling system secured.

Control Tank Recirculation and Discharge

The Control Tank is discharged after the Treatment Tank. The discharge procedure is similar to that of the Treatment Tank. The primary differences include:

- The BWTS is secured, and the 01 Deck by-pass is open.
- The sampling volumes required are less, therefore a different sampling manifold is used.

6.5 Sampling Procedures

These procedures are performed to provide samples to support the analyses described in Section 6.7 – Biological Efficacy Analysis. In addition, these samples are used for the “grab sample” assays described in Section 6.6 – Water Quality Analysis. The below table, Sampling Procedure Coordination, matches the pumping system operation to a particular sampling procedure and the resulting sample replicates and volumes.

Table 6 - Sampling Procedure Coordination

Pumping Operations		Sampling Procedures					
	Rate** (m3/hr)	Name	Location	Pitot/ Size (inches)	Each Replicate		
					Rate** (lpm)	Volume	
						10 - 50 µm	≥50 µm
Uptake							
Treatment (Untreated)	250	3 x 1	A1, A2, A3	S1 (1 x 1.61")	25	3 x 22 liters	3 x 1m3
Control (Untreated)	250	3 x 1	B1, B2, B3	S1 (1 x 1.61")	25	3 x 22 liters	3 x 1m3
Discharge							
Treatment (Treated)	250	3 x 3	A1, A2, A3	S4 (3 x 1.68")	75	3 x 22 liters	3 x 3m3
Control (Untreated)	250	3 x 1	B1, B2, B3	S1 (1 x 1.61")	25	3 x 22 liters	3 x 1m3

**During tank stripping pumping rates slows down.

Sampling Overview

Ballast water sampling procedures vary as per the ballasting operations as follows:

Ballast Water Uptake Untreated Water Samples (3 x 1m3 and 3 x 22 liters).

During ballast water uptake, untreated water is taken from the seachest and moved to the Treatment Tank, and then the Control Tank. During each of these sequential uptakes, three continuous samples of at least 1.0 cubic meter are taken before the BWTS. This method assures sampling during the entirety of both uptake events, and produces two sets of three (3) triplicate samples totaling not less than 3.0 cubic meters for analysis of the ≥50 µm size class, for each uptake.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from the same sample line. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 µm size class.

Ballast Water Discharge Treatment Samples (3 x 3m3 and 3 x 22 liters).

During ballast water discharge from the Treatment Tank, integrated samples of treated water in triplicate of at least 3.0 cubic meters each are taken after the BWTS. This provides a total of three (3) 3.0 cubic meter samples for a total volume of at least 9.0 cubic meters. This method assures sampling during the entire discharge event for analysis of the ≥50 µm size class.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from each of the same sample lines. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 µm size class.

Ballast Water Discharge Control Samples (3 x 1m3).

During ballast water discharge from Control Tank, integrated samples of treated water are taken in triplicate of at least 1.0 cubic meters each. This provides a total of three (3) 1.0 cubic meter samples for a total volume of at least 3.0 cubic meters. This method assures sampling during the entire discharge event for analysis of the ≥50 µm size class.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from each of the three same sample lines. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 µm size class.

Sampling Coordination

Ballast water uptake consists of one event that uptakes to the Treatment Tank (3-154-1) and then immediately uptakes to the Control Tank (3-154-2).

The BWTS inlet piping is primed, and water is pumped through a recirculation loop while the BWTS warms up. When the BWTS is ready, water is pumped to overboard (sea-to-sea). The sea-to-sea mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge from overboard to fill the Treatment Tank.

When the Treatment Tank reaches its designated volume, the team switches to start filling the Control Tank while shutting down the BWTS. When the Control Tank reaches its designated volume, pumping is stopped to clean the piping system of control ballast water.

Ballast water discharge proceeds after ballast water is held for not-less-than 48 hours. Discharge includes two events that discharge the Treatment Tank, followed by the Control Tank.

The first event primes the BWTS inlet piping, starts the BWTS, and takes suction from the Treatment Tank. The treated water passes the BWTS, and returns to the treatment pump suction (recirculation). The recirculation mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge to overboard, emptying the Treatment Tank to sea, while the sampling team takes triplicate sampling of the treated water. The Control Tank is recirculated, now by-passing the BWTS. The untreated control water is then sampled and discharged to sea.

Communications between the pumping team (opening and closing valves, operating the treatment system pump, and operating the BWTS) and the sampling team is performed by radio, visual communications, and the automation system ballast order telegraph (BOT). Of these, the BOT is the primary and definitive means of communication.

The sampling team will prepare the sampling system by flushing all equipment, hoses, and other devices with raw seawater before uptake or held ballasted before discharge. During set-up, the sampling system will also balance flows into the tubs where the plankton nets are rigged. However, the sampling team will only send ballast water through the plankton nets when directed by the pumping team. This is called “netting.” Only during an actual uptake or discharge are the samples netted, in order to maximize the sample representativeness of the ballast tank contents.

6.6 Water Quality Analysis

Continuous Data

In addition to collecting site-specific data, as detailed in the *Facility Preparation* section, Water properties passing the Facility pump will be continuously monitored by the Sea Bird TSG, including temperature, conductivity, salinity, dissolved oxygen, transmissivity, and chlorophyll florescence. These parameters will be logged to the individual instrument, as well as collected and stored in the automation system.

Table 7 – Continuous Monitoring Parameters

Sensor	Maker	Type	Calibration Procedure	Reporting Units	Range
Temperature	Sea-Bird Electronics	SBE 21	Nominally 1/year	degrees C	-5 to +35
Conductivity	Sea-Bird Electronics	SBE 21	Nominally 1/year	S/m	0 to 7
Transmissometer (25 cm)	Wet Labs	C-Star	Nominally 1/year	% transmittance or beam attenuation	0-100
Dissolved Oxygen	Sea-Bird Electronics	SBE 43	Nominally 2/year	mg/L	120% of saturation
Fluorometer	Turner Designs	Cyclops-7	In-house, before each experimental run	ug/L	0.1 to 50

In-Situ Probe Measurements

A handheld hydrographic field meter will be used to measure dissolved oxygen, salinity, and temperature in the biological sampling tanks at the time of sample collection. A bench top pH meter with Ross combination electrode will be used to measure pH. The measurements made here are redundant with those collected continuously by independent sensors associated with the GBF continuous data system.

Grab Samples

Challenge water samples will be taken at the time of ballast uptake to define empirically the chemical and biological characteristics of water entering the control and treatment ballast tanks. These samples will be drawn directly from the uptake pipe flow at the biological sampling station onboard *Golden Bear* using unsieved water, collected in triplicate and with the time-integrated method as described above. Challenge water living organism concentrations will be determined from sieved and unsieved water collections, as described in the above Sampling Procedures – Section 6.5. Table 8 provides sample listings with defined methods. Challenge water sample analyses will include the following:

- Salinity
- Temperature
- pH
- Dissolved Oxygen
- Total Suspended Solids
- Transmittance
- Particulate Organic Carbon
- Dissolved Organic Carbon
- Chlorophyll *a*

Table 8. Challenge water sample parameters and processing details

Parameter	Sample method/ volume	Processing	Sample Storage	Analysis	Notes
Salinity	YSI 6600 handheld probe	immediate	NA	Meter readings, handheld probe unit	Calibrated with Thermo-Fisher conductivity standards
Temperature	YSI 6600 handheld probe	immediate	NA	Meter readings, handheld probe unit	Factory calibrated thermistor probe
Dissolved oxygen	YSI 6600 handheld probe	immediate	NA	Meter readings, handheld probe unit	Calibrated with 100% air saturated water
pH	135 mL polypropylene bottle	Within 6 h of collection	Room °C	Beckman Model 70 pH meter	Two-point pH standardization
Total Suspended Solids (TSS)	2 L polypropylene bottle	Volumetric filtration onto pre-weighed 0.5 µm membrane filter	Dried at 85 °C for 3 hours, stored in vacuum dessicator @ room °C, analyzed within 30 d	Gravimetric weight determination after drying to constant weight, ± 0.1 mg	Weighed on 5-digit balance, granite weighing table (MLML)
Transmittance	20 mL acid-washed glass vial	Water, unfiltered, directly into glass sample container for storage	Room °C	Light propagation analysis to determine beam transmittance.	Readings from 370 nm to 650 nm, at 10 cm and 25 cm.
Particulate Organic Carbon (POC)	2 L polypropylene bottle	Volumetric filtration onto pre-combusted GF/F filter	Dried at 65 °C for 48 h, stored in vacuum dessicator @ room °C, analyzed within 30 d	CHN combustion analysis	Combustion analysis on CEC 440 CHN Analyzer (MLML)
Dissolved Organic Carbon (DOC)	20 mL acid-washed glass vial	Water passed through GF/F filter, directly into glass sample container for storage	Frozen -20 °C, analyzed within 30 d	Catalytic oxidation	Contract analysis with McCampbell Analytical, Inc. (EPA approved), Pittsburg, CA
Chlorophyll a	1 L amber polypropylene bottle	Volumetrically harvested onto 25 mm GF/F filter, immediately extracted in 1.2 mL 90% acetone	90% acetone extracts stored @ -20 °C, analyzed within 30 d	Single-step fluorometric assay for chl <i>a</i> ;; C-8 HPLC for chlorophylls and carotenoids	Turner TD-700 filter fluorometer, calibrated with HPLC-purified authentic chl <i>a</i> standard (Welschmeyer 1990)

6.7 Biological Efficacy Analysis

Biological test procedures for determination of BWTS performance are detailed in this section of the QAPP. Biological testing methods are evolving continuously. There are few routine, time-tested methods for plankton viability determination at this time, such as *Standard Methods*, US EPA, ASTM, or otherwise. Therefore, methods must be devised to meet the quantitative challenges of regulatory performance standards. Detailed biological assays are

included in the Standard Operating Procedures (SOPs). The biological efficacy methods detailed here reflect our desire to:

- Achieve the objectives of the Shipboard study as stated in the Project Plan.
- Utilize instrumentation and facilities at GBF that were assembled specifically for the purpose of viability testing, including development of new methodologies.

The tests for biological efficacy provide numeric counts of living organisms defined either by size category or specific pathogen. Methods for viable organism counts to be used by GBF scientists are detailed below, according to size and pathogen classification. Table 3 summarizes the critical measurements of organism viability made in this study, along with notes on methods and processing techniques.

Section 6.5 describes the collection of samples for these analyses.

Organisms Larger than 50 μm

Organisms larger than 50 μm in minimum physical dimension will be collected with Nitex mesh having a nominal pore size of 50 μm , measured on the diagonal (e.g., 35 μm Nitex plankton nets). Counts of live and dead organisms larger than 50 μm will be made using the so-called ‘poke and probe’ method under 30x (nominal) dissecting microscope observation (see below).

Organisms larger than 50 μm will be collected at each of three replicate sampling stations mounted on the Main Deck of *T/S Golden Bear*. Each sampling station provides metered flow into the mouth of the zooplankton net (three simultaneous replicates). The nets are positioned within large polyethylene containers that retain filtrate water to a user-controlled height, thus bathing the zooplankton net in surrounding water to reduce damage to live captured organisms. Sample water from the appropriate source (e.g., Treatment Uptake, Control Uptake, Treatment Discharge, Control Discharge) will be directed through each net at uniformly metered flow rates to yield continuous samples, integrated over the each ballasting operation. Three replicate 1 m^3 samples will be collected for uptake sampling. Three replicate 3 m^3 samples will be collected for each treatment discharge sampling, while three replicate 1 m^3 samples will be collected for control discharge sampling. The larger collection volume for treatment discharge attempts to improve statistical confidence in samples where the total number of live organisms is expected to be low.

Rates of ca. 25 L/min per each replicate will provide adequate flow to collect zooplankton samples of at least 1 m^3 each during the uptake and control discharge events. The sample flow rate will be increased by a factor of 3x for treatment discharge sampling; output from the calibrated flow meters will be logged continuously by the GBF data acquisition system. Each replicate sampling station will be fit with two nets: one to collect the bulk of the sample, and a spare that can be quickly positioned in-line in case clogging becomes a problem. GBF’s experience indicates that all sample volumes of 1 m^3 will be easily handled by a single net. The large volume treatment tank samples will likely be accommodated by a single zooplankton net, since they will have been filtered (larger than 50 μm) by the BWTS.

Concentrated organisms will be immediately transported to the ship’s laboratory for viability determination by microscopy. The samples will be maintained at ambient water temperature in a darkened, insulated container that is plumbed with flowing surface seawater from the

ship. Cod end contents of the sample nets will be adjusted quantitatively to 500 mL with filtered seawater (0.7 μm GF/F filtrate) and counted immediately. Sample aliquots of 10 mL will be placed in a serpentine counting tray and observed under a stereo microscope; nominally, at ca. 30x magnification for determination of live/dead counts.

All efforts will be made to enumerate live organisms quickly, since the primary metric in ballast treatment performance tests is the determination of live organism counts. Live organisms will be counted first, followed by enumeration of dead organisms in the same sample aliquot. Animals will be designated as 'live' if they are fully intact and actively moving, exhibited an escape response when probed with a fine needle, or showed any internal/external movement. Organisms will be recorded as 'dead' if no activity or movement of any kind was observed, or if organisms were not intact. This viability determination is commonly referred as the 'poke and probe' technique. Totals and fractional portions of live and dead organisms will be tabulated, and all assays will be manipulated volumetrically (if required) so that organism concentrations (number/volume) can be reported. Two separate archive samples will be preserved in 4% buffered formalin, to allow for the inspection of general taxonomic diversity under less pressing time constraints, and kept for at least six (6) months.

Organisms Larger than 10 μm , but Smaller than 50 μm – Required Assays

It is widely recognized that the absolute numerical determination of viable unicellular protists in the size range 10 μm to 50 μm is ambiguous (Dobroski 2009). This follows from the fact that visible signs in distinguishing live from dead protists are usually not evident (except in the case of motile representatives). For this reason, several corroborative methods will be used on board ship to evaluate the diverse assemblage of microbiota occurring in the size range larger than 10 μm but smaller than 50 μm . The methods will include techniques that specifically yield estimates of viable cell concentrations. Whole water (unsieved) for use in preparing the 10 to 50 μm samples will be collected in 20 L carboys, as described in Section 3.

GBF will use a size fractionation technique to isolate the organisms smaller than 50 μm , but larger than 10 μm for all assays listed below. This is easily accomplished by concentrating the smaller than 50 μm filtrate (passed through a 50 μm sieve) on to custom made Nitex filters of 10 μm pore size on the diagonal (Nitex product 03/7-2) and re-suspending the retained particles for direct use in the assays below. GBF has been able to concentrate samples ten to one hundred times greater than ambient concentrations utilizing this method; this significantly increases the analytical sensitivity and precision of techniques used to measure low concentrations of viable organisms expected in treatment samples. Two separate archive samples will be preserved in 1% glutaraldehyde, to allow for the inspection of general taxonomic diversity under less pressing time constraints, and kept for at least six (6) months.

Chlorophyll-based most probable number (MPN) determination of viable cell concentration – Required Assay

Photoautotrophic growth will be measured from long term incubations (14d) by using whole-cell chlorophyll fluorescence analysis as a sensitive indicator of cellular growth. MPN culture arrays of serially-diluted sample water will be prepared with F/2 seawater media (adjusted for ballast water salinity) in clear micro-tubes (0.5 mL volume) that can be read directly in a Spex

Fluoromax 2 spectrofluorometer on board ship. The MPN array will be constructed to yield optimized detection of 10 living organisms per mL (Woomer et al., 1990). Nominally the MPN culture arrays will include 25 tubes per sample (5 replicates x 5 dilution levels). MPN cultures will be maintained in an illuminated incubator at 5°C above natural temperature (to promote rapid growth) and monitored every other day for fluorometric indication of chlorophyll growth, defined as a two-fold increase in chlorophyll fluorescence relative to values scored at time zero. Triplicate MPN arrays will be set up for each sample, thus dictating the use of small growth tubes to conserve space within the incubator for all samples acquired from the three separate ballasting test cycles described here. One scientific technician will remain onboard ship after the last ballast test cycle to complete the daily fluorometric analysis of MPN arrays through the full 14d incubation period for each sample.

C-14 Primary Production Experiments – Required Assay

The radiotracer C-14 technique will be applied to uptake, control and treatment samples (each in triplicate) to yield physiological measurements of photosynthesis (carbon fixation rates). Experiments will be initiated and terminated at MLML in a walk in cold room nominally held at 13 °C. Samples will be prepared in triplicate, acid-washed polycarbonate bottles (125 mL), inoculated with C-14 (2 µCi) and incubated for 24 hours under continuous, constant illumination provided by high intensity LED lamps; bottles will be rotated continuously on motorized plankton wheels to ensure uniform irradiance exposure and to prevent settling of cells. C-14 processing will follow that of Welschmeyer et al. (1993). Whole water sample aliquots will be harvested onto GF/F filters (0.7 µm) and 10 µm nylon filters to estimate total and >10 µm photosynthetic rates, respectively (µgC L⁻¹ d⁻¹). Total dissolved inorganic carbonate (DIC) will be determined on a UIC CM5012 CO₂ Coulometer for proper determination of DIC specific activity (dpm/gC). Chlorophyll specific photosynthetic rates will be computed from Chl measurements made on the same water samples.

Organisms Larger than 10 µm, but Smaller than 50 µm – Corroborative Assays

The previously described *Required Assays* are complete for the 10 – 50 µm size class as specified by the IMO G8 Guidelines that this test plan must comply with. This test plan is not required to comply with the newly released ETV Protocol that includes additional testing requirements for the 10 – 50 µm size class. As such, these additional tests are listed here as optional Corroborative Assays.

Whole water (unsieved) for use in preparing the 10 to 50 µm samples will be collected in 20 L carboys, as described in Section 3.

It is hoped that the use of independent and corroborative methods will add confidence in the efficacy testing for the problematic 10 to 50 µm size class.

Flow cytometric analysis of live cells utilizing fluorescein diacetate (FDA) vital stain – Corroborative Assay

GBF will maintain a Becton-Dickinson FACScan flow cytometer on board ship throughout the two-three month experimental period to be used for quantitative analysis of living cell concentrations utilizing FDA vital stain protocol (Geary et al 1997; Hayakawa et al. 2008). FDA is a colorless reagent that freely passes through cell membranes and, when acted upon

by living cellular esterase activity, is converted to the brilliant green fluorescent product, fluorescein, which readily marks viable cells for flow cytometric detection. GBF will focus primarily on the detection of larger phytoplankton cells, as opposed to colorless heterotrophs, since phytoplankton provide natural red chlorophyll fluorescence; this yields a robust, two-color discrimination (red/green) for quantitative cytometry. The cytometric technique depends on the detection of obvious cell populations using optical scattering and fluorescence signals. GBF will rely on natural 'red' chlorophyll fluorescence to determine natural phytoplankton population targets, as this provides the optimal optical discrimination to identify the 'green' fluorescent live cells after the addition of FDA. Inert fluorescent bead standards of 10 μm and 50 μm will be used to roughly establish the cytometric region of analysis (based on forward scatter), thus allowing us to gate out the more numerous, small cells (smaller than 10 μm) that are sure to be present in all samples.

Visual epifluorescence detection of viable 10 to 50 μm cells utilizing FDA and CMFDA tracers – Corroborative Assay

Visual enumeration of live cells in the 10 to 50 μm size category will be made using similar protocol to that described for flow cytometry (4.2.2.2). In this case, two fluorescent markers, FDA (fluorescein diacetate) and CMFDA (chloromethylfluorescein diacetate), will be applied simultaneously to maximize visual fluorescent signals and to minimize color fade during the counting procedure. FDA and CMFDA will be added to a final concentration of 5 μM and 2.5 μM , respectively, and incubated for 10 minutes before mounting in a covered 1 mL counting chamber for epifluorescence enumeration utilizing blue excitation and green emission.

Thus, three methods for enumerating viable cells in the size class smaller than 50 μm but larger than 10 μm will be used:

- MPN culture by chlorophyll detection.
- Viability staining with FDA, flow cytometric detection.
- Viability staining with FDA and CMFDA.

It is hoped that the use of independent and corroborative methods will add confidence in the efficacy testing for the problematic 10 to 50 μm size class.

Organisms Less than 10 μm

Five assays will be used in the detection of living organisms in the smallest size class, less than 10 μm . The first is the bulk assay for total cultivable bacteria and the remaining four assays are directed to specific microbial pathogens, *Escherichia coli*, *Enterococci sp.*, *Vibrio cholerae* serotype 01, and *V. cholerae* serotype 0139.

Bulk heterotrophic bacteria plate counts

Traditional sterile plating technique will be used to enumerate colony forming units (CFU) of the bulk bacterial community passing through a 10 μm sieve. Volumetric (100 μL) sample aliquots (in triplicate) will be spread on sterile marine agar plates (Difco, 100 mm dia.) and incubated overnight (maximum of 24 hours) under dark conditions and at room temperature. Plates will be photographed in a digital image analyzer (Bio Rad Fluor S-Max) and

enumerated using colony-counting software provided with the instrument. Data will be tabulated as CFU/mL.

Microbial pathogens

Test kits based on quantitative colony forming unit (CFU) measurements specific to *E. coli* and *Enterococci* will be used on board ship using sterile protocol. *E. coli* and *Enterococci* will be assayed using the Colilert[®] and Enterolert[®] test kits (Idexx, Inc.), which are based on MPN methodology and species-specific chromogenic reactions. Both assays will be prepared in triplicate using heat sealed dilution trays (Quanti-Tray[®] Idexx, Inc.) incubated at 35 °C for 24 h. *V. cholera* will be assayed using test kits for the 01 and 0139 serotypes, Cholera Smart[®] II and Bengal Smart[®] II, respectively (New Horizons Diagnostics, Inc.). The kits were originally produced for the purpose of obtaining quick (20 min) Cholera presence/absence tests in fecal stool samples; this method is rapid, but relatively insensitive. We have worked with New Horizons (Larry Loomis, CEO) to show that tolerance limits for Cholera of less than 1 CFU/100 mL can be achieved with a 48 h, 35°C incubation using Cholera Smart kits as packaged by the manufacturer (determined from quantitative dilutions of actively growing cultures of *V. cholerae* 01 and 0139 serotypes). GBF will use the prolonged incubation for *V. cholera* to achieve positive/negative scores at less than 1 CFU mL. All of the microbial assays above will be completed in triplicate, and all waste solutions will be bleach-sterilized before disposal.

Section 7 Assessments

As per the Project Plan, the Verification Organization for this project will audit project documentation and performance for compliance with the TQAP and the IMO G8 Guidelines.

Appendix A Project Training Requirements

Quality is responsible to ensure that each person is trained as appropriate to their tasks on the project, as follows:

- Facility Training:** This training covers all information provided in the TQAP, along with specialized training relevant to their role per the test plan; i.e., Confined Spaced Entry Procedures and Documentation, Safety Reviews, etc.
- Equipment Training:** This training covers all information provided by the equipment manufacturer, along with specialized training relevant to the role in testing; i.e., System Installation Requirements Review (particularly US Coast Guard and ABS requirements for electrical connections and ground protection), Lock-Out/Tag-Out, etc.
- SOP Training:** This training ensures that personnel can successfully complete their assigned tasks using the appropriate SOP.

Quality is also responsible for documenting and maintaining training program records. The Director, Lead Operator and Lead Scientist will conduct the training unless otherwise designated by Quality. The logs on the following pages are for documentation of the project training completed by all GBF personnel before the start of testing. A copy of these training records will be filed on board the GBF, and a summary will be included in the final testing report.

Training Logs

Ballast Facility: William Davidson is the chief engineer of the *Training Ship GOLDEN BEAR* and has been the lead engineer during the construction of the GBF and has become expert with all of the technical aspects of the system. Bill is the trainer for those GBF personnel who operate the pumps, valves, etc.

List of personnel trained to operate the ballast pump and valve system:

- Dan Lintz, trained November 2010
- John Coyle, trained November 2010
- Dan Weinstock, trained May 2010 and updated November 2010
- David Coleman, trained May 2010 and updated November 2010
- Bill Schmidt, trained July 2010 and updated November 2010

Water Quality: Richard Muller has installed and maintained in situ water quality sensors for a good part of his career as a technician onboard various oceanographic research vessels during the past 20 years. His training has been primarily through manufacturer interaction and at-sea experience troubleshooting instrumentation.

Biological:

Dr. Nicholas Welschmeyer is professor and research scientist at the Moss Landing Marine Laboratories and has performed research on ballast water and ballast treatment systems for the past seven years with 31 years of field experience in biological oceanography. All biological analysts are trained within the laboratory of Nicholas Welschmeyer. First they are given the full set of standard operating procedures verifying that they have a command of the techniques. Second, for subjective analyses, analysts count trial samples simultaneously to determine comparability between analysts. Third, all biological analysts are familiarized with instrumentation used for ETV testing.

List of scientific technicians are:

- Erin Jensen
- Julie Kuo
- Brian Maurer
- Jeff Johnsen

Log Sheet 1: Facility Training

[illegible]

Log Sheet 2: Equipment Training

[illegible]

Log Sheet 3: SOP Training

[illegible]

Test Quality Assurance Plan – Project Charlie

Volume IV: Standard Operating Procedures (SOPs)

Golden Bear Facility
Vallejo, California

Dept. 76408, Fund 46408
9 April 2012, Rev. A



Preface

These Standard Operating Procedures (SOPs) are generic to the testing of a combination filtration and ultraviolet radiation UV ballast water treatment system (BWTS) at the Golden Bear Facility (Facility). These procedures adhere to the international guidelines provided in MEPC.174(58) *Guidelines for Approval of Ballast Water Management Systems (G8)*.

These SOPs were also developed to comply with the requirements of the U.S. EPA Environmental Technology Verification Program *Generic Protocol for the Verification of Ballast Water Treatment Technology (ETV)*. However, as ETV does not yet certify facilities, it is not possible to assure that testing performed to these SOPs will be retroactively accepted. As such, compliance with G8 requirements takes precedence. SOPs that are only in support of ETV requirements and not required by G8 requirements are designated as corroborative.

Project Charlie refers to a single UV-BWTS that is scheduled for testing at the Facility. This testing is governed by a Test Quality Assurance Plan (TQAP) that consists of these SOPs, a Project Plan (PP), and two Quality Assurance Project Plans (QAPP), one for Shipboard testing and one for Land-Based testing. Information on the Project Charlie BWTS can be found in the PP and QAPPs. Finally, all Facility activities are governed by the Facility Quality Management Plan (QMP). Wherever a conflict exists between documents, these SOPs takes first precedence and the QAPPs take second precedence.

Facility Nomenclature Breakdown

The facility uses specific nomenclature to describe various activities. The following provides definitions, and applies this nomenclature to Project Charlie.

Project. A Project is a collection of tests to verify a technology claim, or collection of experiments to prove a thesis. Project Charlie consists of five brackish and five salt water tests to verify that the BWTS meets the IMO D-2 standard. The project will be performed in accordance to the IMO G8 Guidelines for Shipboard and Land-Based testing, and the ETV Land-Based protocol.

Test. A Test is one replicate activity that compares the performance of a treatment system (or other method) to a control. Each Project Charlie Test consists of a sequential treatment uptake through the treatment system and a control uptake with no treatment. These two separate ballast water parcels have similar challenge conditions as they are taken from the same ambient water source (Shipboard) or the same “source tanks” (Land-Based). The two parcels are held separately in a “treatment” tank and a “control” tank. The treatment is discharged through the treatment system after a designated hold time. The control is discharged without treatment after a similar hold time.

Event and Cycle. The Facility uses the term Event to describe a discrete combination of activities, typically performed without stopping, resetting, or other breaks. It typically takes several Events to complete a Test. The Facility uses the term Cycle to describe a series of events that accomplishes two tests.

Project Charlie Testing Sequence

Preparation

Preparation includes the following Events:

- BWTS Commissioning
- BWTS Stress Testing
- Tank Inspections

Shipboard Tests

Four (4) total Tests completed, at separate locations with different ambient water conditions.

Test 1. Uptake Oakland, CA. Discharge Vallejo, CA. Test 1 includes the following events:

- Initial Tank Cleaning
- Pipe Cleaning
- Treatment Uptake
- Control Uptake
- Pipe Cleaning
- Treatment Discharge
- Control Discharge

Test 2. Uptake Sacramento, CA. Discharge at sea. Test 2 includes the following events:

- Pipe Cleaning
- Treatment Uptake
- Control Uptake
- Pipe Cleaning
- Treatment Discharge
- Control Discharge

Test 3. Uptake Los Angeles, CA. Discharge at sea. Test 3 includes the following events:

- Pipe Cleaning
- Treatment Uptake
- Control Uptake
- Pipe Cleaning
- Treatment Discharge
- Control Discharge

Test 4. Uptake Vallejo, CA. Discharge Oakland, CA. Test 4 includes the following events:

- Pipe Cleaning
- Treatment Uptake
- Control Uptake
- Pipe Cleaning
- Treatment Discharge
- Control Discharge

Land-Based Brackish Water Tests

Six (6) total Tests completed in three Cycles.

Cycle A. Consists of Tests 1 and 2. Cycle A includes the following events:

- Tank and Pipe Cleaning
- Test 1 Treatment Uptake and Combined Test 1 and 2 Control Uptake (Note: a single control parcel serves two treatment Tests)
- Pipe Cleaning
- Test 2 Treatment Uptake
- Pipe Cleaning
- Test 1 Treatment Discharge
- Test 2 Treatment Discharge
- Combined Test 1 and 2 Control Discharge

Cycle B. Consists of Tests 3 and 4. Cycle B includes the following Events:

- Tank and Pipe Cleaning
- Test 3 Treatment Uptake and Combined Test 3 and 4 Control Uptake
- Pipe Cleaning
- Test 4 Treatment Uptake
- Pipe Cleaning
- Test 3 Treatment Discharge
- Test 4 Treatment Discharge
- Combined Test 3 and 4 Control Discharge

Cycle C. Consists of Tests 5 and 6. Cycle C includes the following Events:

- Tank and Pipe Cleaning
- Test 5 Treatment Uptake and Combined Test 5 and 6 Control Uptake
- Pipe Cleaning
- Test 6 Treatment Uptake
- Pipe Cleaning
- Test 5 Treatment Discharge
- Test 6 Treatment Discharge
- Combined Test 5 and 6 Control Discharge

Land-Based Marine Water Tests

Six (6) total Tests completed in three Cycles.

Cycle D. Consists of Tests 7 and 8. Cycle D includes the following Events:

- Tank and Pipe Cleaning
- Test 7 Treatment Uptake and Combined Test 7 and 8 Control Uptake
- Pipe Cleaning
- Test 8 Treatment Uptake
- Pipe Cleaning
- Test 7 Treatment Discharge
- Test 8 Treatment Discharge
- Combined Test 7 and 8 Control Discharge

Cycle E. Consists of Tests 9 and 10. Cycle E includes the following Events:

- Tank and Pipe Cleaning
- Test Nine Treatment Uptake and Combined Test 9 and 10 Control Uptake
- Pipe Cleaning
- Test 10 Treatment
- Pipe Cleaning
- Test 9 Treatment Discharge
- Test 10 Treatment Discharge
- Combined Test 9 and 10 Control Discharge

Cycle F. Consists of Tests 11 and 12. Cycle E includes the following Events:

- Tank and Pipe Cleaning
- Test 11 Treatment Uptake and Combined Test 11 and 12 Control Uptake
- Pipe Cleaning
- Test 12 Treatment Uptake
- Pipe Cleaning
- Test 11 Treatment Discharge
- Test 12 Treatment Discharge
- Combined Test 11 and 12 Control Discharge

Standard Operating Procedures – UV Based Systems

Procedures Performed Once per Project

- SOP 1 Quality Control Checklist For Project
- SOP 2 Posted Placards and Piping Line-Ups (Land-Based)
- SOP 3 Posted Placards and Piping Line-Ups (Shipboard)
- SOP 4 BWTS Commissioning
- SOP 5 BWTS Shakedown Test

Procedures Performed Each Test Cycle (Uptake, Holding, and Discharge)

- SOP 6 Quality Control Checklist for Cycle (Land-Based)
- SOP 7 Quality Control Checklist for Cycle (Shipboard)
- SOP 8 Tank Cleaning (Land-Based)
- SOP 9 Tank Cleaning (Shipboard)
- SOP 10 Preparation of Source Tanks (Land-Based)
- SOP 11 Calibration of Sampling System Flow Meters
- SOP 12 Calibration of Sea-Bird Thermosalinograph (TSG)
- SOP 13 Calibration of Flow Cytometer & Lab Fluororometer
- SOP 14 Operation of BWTS and Facility Piping System (Land-Based)
- SOP 15 Operation of BWTS and Facility Piping System (Shipboard)
- SOP 16 Operation of Ballast Water Sampling System (Land-Based)
- SOP 17 Operation of Ballast Water Sampling System (Shipboard)
- SOP 18 Chain of Custody Record (SOPs xx – xx)

Biological Analysis - Required

- SOP 19 Biological Analysis Data Sheets (SOPs 20, 23, 27)
- SOP 20 Poke and Probe Viability Determination for Organisms $\geq 50 \mu\text{m}$

Standard Operating Procedures – UV Based Systems

- SOP 21 Most Probable Number (MPN) Determination of Viable Phytoplankton Cells ≥ 10 to < 50 μm , Chlorophyll-based
- SOP 22 C-14 Primary Production
- SOP 23 Chlorophyll *a*
- SOP 24 Heterotrophic Bacteria Plate Counts for Organisms < 10 μm
- SOP 25 Indicator Microbes *E. coli* and *Enterococci* for Organisms < 10 μm
- SOP 26 Indicator Microbes *Vibrio Cholerae* Serotype 01 and *Vibrio Cholerae* Serotype 0139 for Organisms < 10 μm

Biological Analysis - Corroborative

- SOP 27 FDA-Based, Flow Cytometric Analysis of Viable Organisms ≥ 10 μm but < 50 μm
- SOP 28 FDA/CMFDA Epifluorescence Analysis of Viable Organisms ≥ 10 μm but < 50 μm

Water Chemistry Analysis

- SOP 29 In-situ Thermosalinograph (TSG) Operation, Data Collection, and Data Processing
- SOP 30 In-situ Probe Measurements: Dissolved Oxygen, Salinity, Temperature, pH
- SOP 31 Grab Sample – Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON)
- SOP 32 Grab Sample – Total Suspended Solids (TSS)
- SOP 33 Grab Sample – Transmittance
- SOP 34 Grab Sample – Dissolved Organic Carbon (DOC)
-

SOP 1 Quality Control Checklist for Project

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as it is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge:

Name/Title

Sign

Date

Quality Officer:

Name/Title

Sign

Date

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

SOP 1, Step 1 Red Line Procedures

As testing efforts progress, the complexity of the operations and variability of the equipment and challenge water conditions, it may at times be advantageous or imperative to change procedures.

In any case, where there is a deviation from the test procedures, protocol, plan, etc. the person-in-charge of that operation must “red-line” that document. Redlining is accomplished by:

- Strike a single line, using indelible ink, through the affected text, figure, chart, or other item.
- Initial each adjacent to each and every strike.
- Provide, using indelible ink, any required correction in the form of text, figure, chart, or other item.
- Provide an explanation in the “notes” section of why the change was made.

Date and sign each affected page.

SOP 1, Step 2 Documentation Distribution

Initial each completed item.

- Post Test Placards (SOP 2) at Pumping Station, Sampling Station, and Laboratory
 - Emergency Contact Numbers _____.
 - Valid Test Parameters _____.
 - Sample and Pumping Rate Coordination _____.
 - Sampling Rates _____.
- Distribute approved Facility and Project documents listed below _____.

Documentation Distribution Table

Document Information	Distribution				
	Director	Admin	Ops	Science	
Name					Notes
Facility Documents					
EH&S Plan	X	X	X	X	
QMP	X	X	X	X	
Facility Description	X	X	X	X	
Project Specific Documents					
Project Plan	X	X	X	X	
QAPP – Shipboard	X	X	X	X	
QAPP – Land-Based	X	X	X	X	
Standard Operating Procedures	X	X	X	X	

Notes: _____

SOP 2 Posted Placards and Piping Line-Ups (LAND-BASED)

Application	LAND-BASED	XX	SHIPBOARD
-------------	------------	----	-----------

(Placard – Post at Pumping Station, Sampling Station, and Laboratory)

Emergency Contacts (listed in notification order)

- William T. Davidson, Facility Director**
Golden Bear Facility
707.654.1304, cell 707.592.4267
bdavidson@csum.edu
- Harry Bolton, Ship Master**
California Maritime Academy
707.654.1192, cell 707.246.7495
HBolton@csum.edu
- Cal Maritime Emergency Operations Center**
707.654.1111
- MLML ES&H Contacts**
Moss Landing Marine Laboratories
831.771.4400
- Jocelyn Douglas, Health and Safety Officer**
831.771.4451, cell 831.750.9563

Additional Project Contacts

Richard S. Muller, Quality Officer
Golden Bear Facility
707.654.1258
rmuller@csum.edu

Veronica Boe, Facilities Program Support
California Maritime Academy
707.654.1156
vboe@csum.edu

Nicholas A. Welschmeyer, PhD, Lead Scientist
Golden Bear Facility
831.771.4439
welschmeyer@mlml.calstate.edu

Jad Mouawad, Senior Engineer
Det Norske Veritas
+47 480 50 902
jad.mouawad@dnv.com

Andrew Daley, Project Manager
Trojan UV
519.457.3400, cell 226.688.7430
adaley@trojanuv.com

Test Cycle Number

PIC Initials

Quality Initials

Date

(Placard – Post at Pumping Station, Sampling Station, and Laboratory)

	Criteria		Uptake Events			Discharge Events		
			Treat 1	Treat 2	Control	Treat 1	Treat 2	Control
Treatment Line and Tanks								
Average (m³/hr)	250 ± 10%							
Volume at end Cycle (m³)	200 (-0%/+10%)							
Control Line and Tank								
Total Volume (m³)	200 (-0%/+10%)							
Combined Sample Volume (m³)								
Uptake	≥ 1							
Control Discharge	≥ 3							
Treatment Discharge	≥ 9							
Ballast Hold Duration (hours)	120 (-0%/+10%)							
Water Quality	Brackish	Salt						
Salinity (PSU)	10 - 20	>32						
Temperature (Celcius)	4 - 35	4 - 35						
DOC (mg/L)	≥ 6	≥ 6						
POC (mg/L)	≥ 5	≥ 4						
TSS (mg/L)	≥ 50	≥ 24						
Uptake Living Populations								
≥50 microns (organisms/m³)	10^5							
≥10 < 50 microns (organisms/mL)	10^3							
<10 microns (bacteria/mL)	10^4							
Control Living Populations								
≥50 microns (organisms/m³)	100							
≥10 < 50 microns (organisms/mL)	100							
<10 microns (bacteria/mL)	500							

(Placard – Post at Pumping Station, Sampling Station, and Laboratory)

		Treatment Uptake	Control Uptake	Treatment Discharge	Treatment Stripping	Control Discharge	Control Stripping	Treat Uptake
Pumping Rate	(m3/hr)	250	250	250	50	250	50	T-0
Sample Port Location		S-1	S-1	S-4	S-4	S-1	S-1	S-2
Pitot Tube I.D.	(inch)	1.05	1.05	3x1.68	3x1.68	1.61	1.61	0.58
Sample Tub Set		B	A	A	A	B	B	170L
Sample Rate Average	(gpm)	5.9	5.9	3x19.8	3x3.64	3x6.64	3x1.33	
Sample Volume Target	(m3)	1.1	1.1	3x3.6	**	3x1.2	**	

Flow Rates for 1.1m3 Sample w/ 1.05 Inch I.D. Pitot at 48 Minutes

Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate/ MagFlo Sensor	1.2	5.6	5.9	6.1

Flow Rates for 10.8m3 Sample w/ 3x1.68 Inch I.D. Pitots at 48 Minutes

Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate per Pitot/ Sample to Tanks (gpm)	4.0	19.0	19.8	20.6

Throttle Flow Meters as per Table

Tub 1 Rate (gpm)*	3.97	19.04	19.83	20.63
Tub 2 Rate (gpm)*	3.97	19.04	19.83	20.63
Tub 3 Rate (gpm)*	3.97	19.04	19.83	20.63

Flow Rates for 3.6m3 Sample w/ 1.61 Inch I.D. Pitot at 48 Minutes

Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate/ Sample to Manifold (gpm)	4.0	19.1	19.9	20.7

Throttle Flow Meters as per Table

Tub 1 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 2 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 3 Rate (gpm)*	1.33	6.37	6.64	6.90

Test Cycle Number

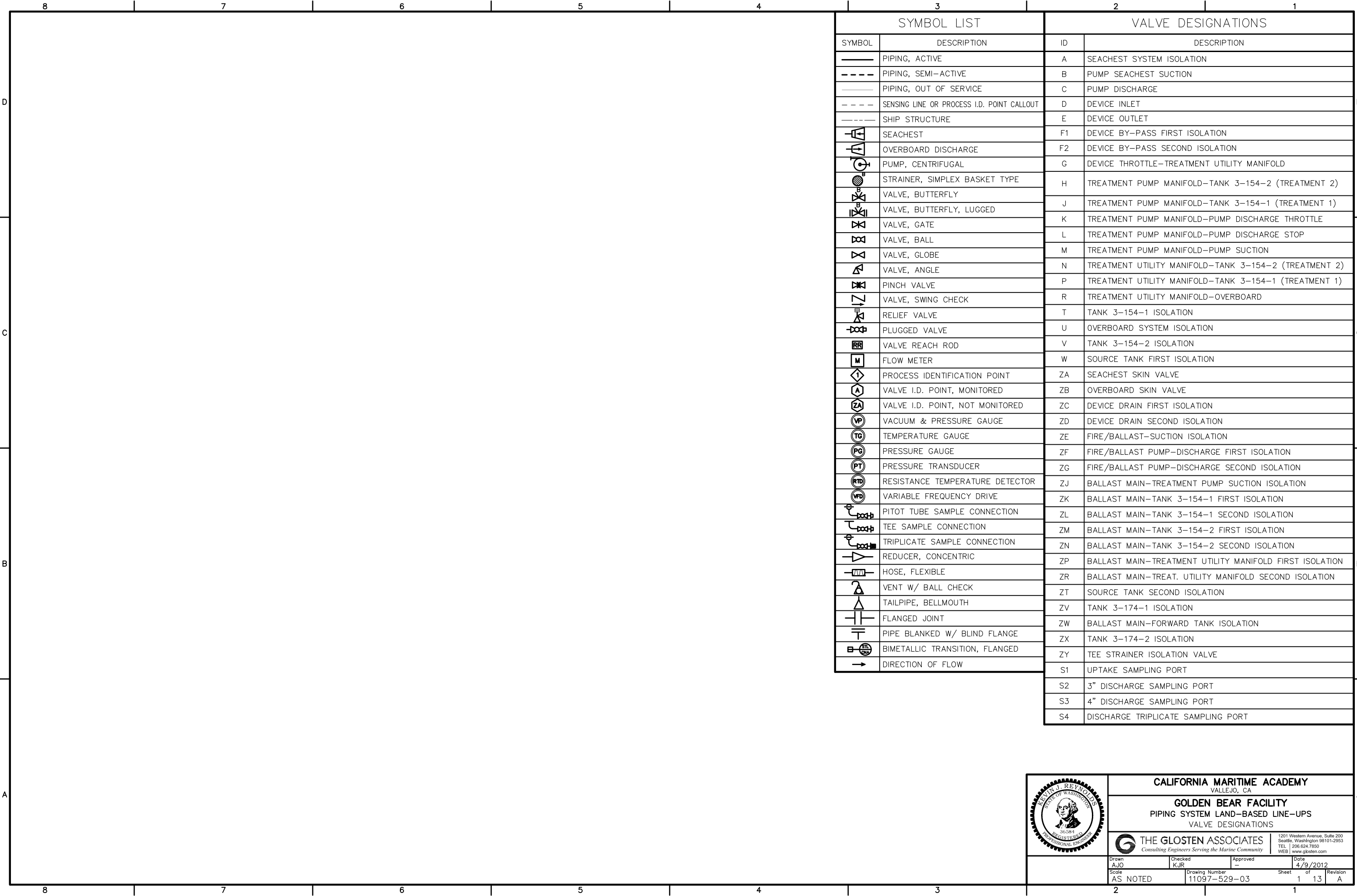
PIC Initials

Quality Initials


Date

SOP 2 (Continued)

The following diagrams provide illustrations of the piping system line-ups.




SYMBOL LIST		VALVE DESIGNATIONS	
SYMBOL	DESCRIPTION	ID	DESCRIPTION
	PIPING, ACTIVE	A	SEACHEST SYSTEM ISOLATION
	PIPING, SEMI-ACTIVE	B	PUMP SEACHEST SUCTION
	PIPING, OUT OF SERVICE	C	PUMP DISCHARGE
	SENSING LINE OR PROCESS I.D. POINT CALLOUT	D	DEVICE INLET
	SHIP STRUCTURE	E	DEVICE OUTLET
	SEACHEST	F1	DEVICE BY-PASS FIRST ISOLATION
	OVERBOARD DISCHARGE	F2	DEVICE BY-PASS SECOND ISOLATION
	PUMP, CENTRIFUGAL	G	DEVICE THROTTLE-TREATMENT UTILITY MANIFOLD
	STRAINER, SIMPLEX BASKET TYPE	H	TREATMENT PUMP MANIFOLD-TANK 3-154-2 (TREATMENT 2)
	VALVE, BUTTERFLY	J	TREATMENT PUMP MANIFOLD-TANK 3-154-1 (TREATMENT 1)
	VALVE, BUTTERFLY, LUGGED	K	TREATMENT PUMP MANIFOLD-PUMP DISCHARGE THROTTLE
	VALVE, GATE	L	TREATMENT PUMP MANIFOLD-PUMP DISCHARGE STOP
	VALVE, BALL	M	TREATMENT PUMP MANIFOLD-PUMP SUCTION
	VALVE, GLOBE	N	TREATMENT UTILITY MANIFOLD-TANK 3-154-2 (TREATMENT 2)
	VALVE, ANGLE	P	TREATMENT UTILITY MANIFOLD-TANK 3-154-1 (TREATMENT 1)
	PINCH VALVE	R	TREATMENT UTILITY MANIFOLD-OVERBOARD
	VALVE, SWING CHECK	T	TANK 3-154-1 ISOLATION
	RELIEF VALVE	U	OVERBOARD SYSTEM ISOLATION
	PLUGGED VALVE	V	TANK 3-154-2 ISOLATION
	VALVE REACH ROD	W	SOURCE TANK FIRST ISOLATION
	FLOW METER	ZA	SEACHEST SKIN VALVE
	PROCESS IDENTIFICATION POINT	ZB	OVERBOARD SKIN VALVE
	VALVE I.D. POINT, MONITORED	ZC	DEVICE DRAIN FIRST ISOLATION
	VALVE I.D. POINT, NOT MONITORED	ZD	DEVICE DRAIN SECOND ISOLATION
	VACUUM & PRESSURE GAUGE	ZE	FIRE/BALLAST-SUCTION ISOLATION
	TEMPERATURE GAUGE	ZF	FIRE/BALLAST PUMP-DISCHARGE FIRST ISOLATION
	PRESSURE GAUGE	ZG	FIRE/BALLAST PUMP-DISCHARGE SECOND ISOLATION
	PRESSURE TRANSDUCER	ZJ	BALLAST MAIN-TREATMENT PUMP SUCTION ISOLATION
	RESISTANCE TEMPERATURE DETECTOR	ZK	BALLAST MAIN-TANK 3-154-1 FIRST ISOLATION
	VARIABLE FREQUENCY DRIVE	ZL	BALLAST MAIN-TANK 3-154-1 SECOND ISOLATION
	PITOT TUBE SAMPLE CONNECTION	ZM	BALLAST MAIN-TANK 3-154-2 FIRST ISOLATION
	TEE SAMPLE CONNECTION	ZN	BALLAST MAIN-TANK 3-154-2 SECOND ISOLATION
	TRIPPLICATE SAMPLE CONNECTION	ZP	BALLAST MAIN-TREATMENT UTILITY MANIFOLD FIRST ISOLATION
	REDUCER, CONCENTRIC	ZR	BALLAST MAIN-TREAT. UTILITY MANIFOLD SECOND ISOLATION
	HOSE, FLEXIBLE	ZT	SOURCE TANK SECOND ISOLATION
	VENT W/ BALL CHECK	ZV	TANK 3-174-1 ISOLATION
	TAILPIPE, BELLMOUTH	ZW	BALLAST MAIN-FORWARD TANK ISOLATION
	FLANGED JOINT	ZX	TANK 3-174-2 ISOLATION
	PIPE BLANKED W/ BLIND FLANGE	ZY	TEE STRAINER ISOLATION VALVE
	BIMETALLIC TRANSITION, FLANGED	S1	UPTAKE SAMPLING PORT
	DIRECTION OF FLOW	S2	3" DISCHARGE SAMPLING PORT
		S3	4" DISCHARGE SAMPLING PORT
		S4	DISCHARGE TRIPPLICATE SAMPLING PORT



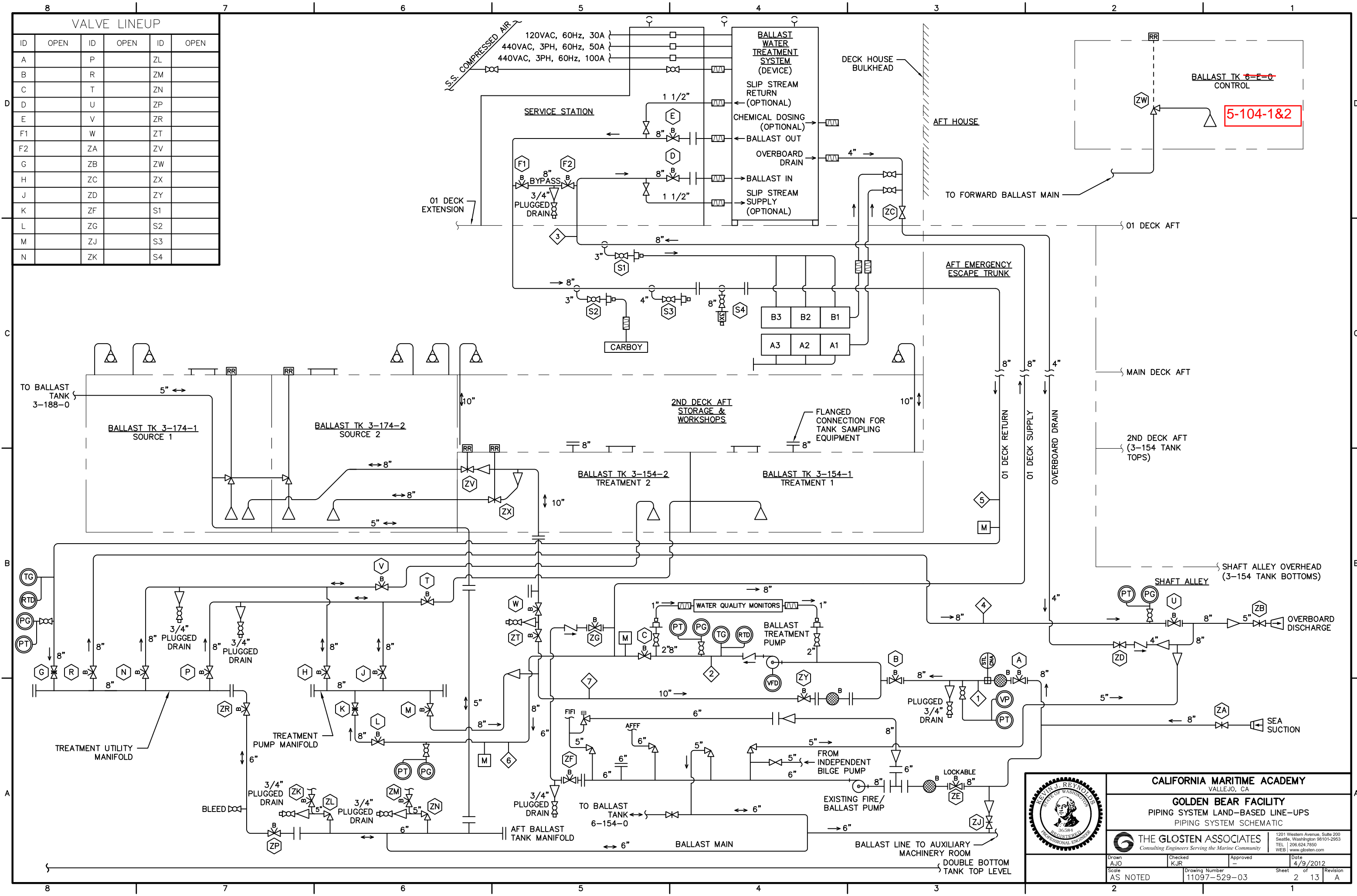
CALIFORNIA MARITIME ACADEMY
VALLEJO, CA

GOLDEN BEAR FACILITY
PIPING SYSTEM LAND-BASED LINE-UPS
VALVE DESIGNATIONS

**THE GLOSTEN ASSOCIATES**
Consulting Engineers Serving the Marine Community

1201 Western Avenue, Suite 200
Seattle, Washington 98101-2953
TEL | 206.624.7850
WEB | www.glosten.com

Drawn AJO	Checked KJR	Approved	Date 4/9/2012
Scale AS NOTED	Drawing Number 11097-529-03	Sheet 1 of 13	Revision A



VALVE LINEUP					
ID	OPEN	ID	OPEN	ID	OPEN
A		P		ZL	
B		R		ZM	
C		T		ZN	
D		U		ZP	
E		V		ZR	
F1		W		ZT	
F2		ZA		ZV	
G		ZB		ZW	
H		ZC		ZX	
J		ZD		ZY	
K		ZF		S1	
L		ZG		S2	
M		ZJ		S3	
N		ZK		S4	



CALIFORNIA MARITIME ACADEMY
VALLEJO, CA

GOLDEN BEAR FACILITY
PIPING SYSTEM LAND-BASED LINE-UPS
PIPING SYSTEM SCHEMATIC

THE GLOSTEN ASSOCIATES
Consulting Engineers Serving the Marine Community

Drawn
AJO

Checked
KJR

Approved

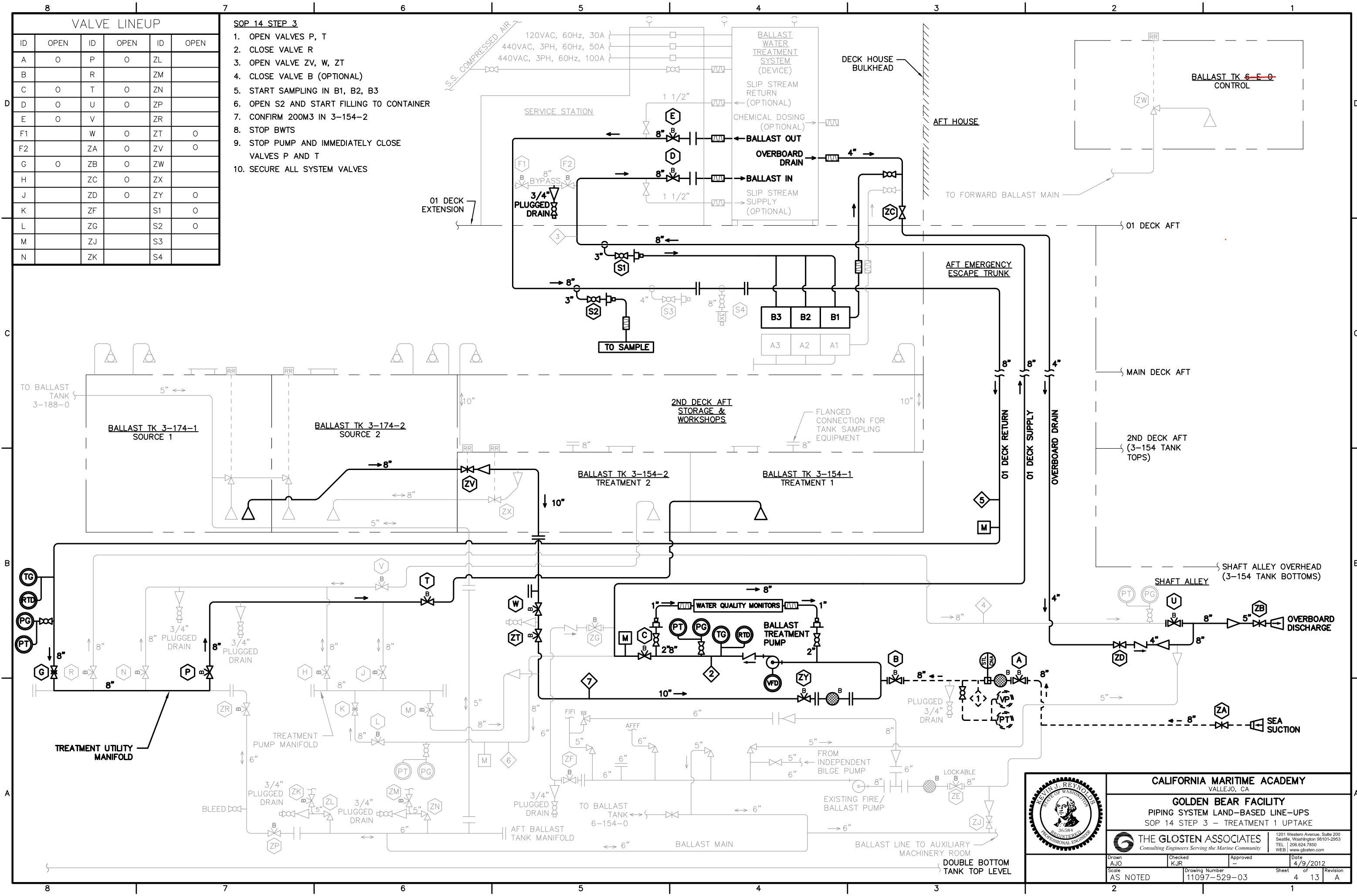
Date
4/9/2012

Scale
AS NOTED

Drawing Number
11097-529-03

Sheet
2 of 13

Revision
A



KEVIN J. REYNOLDS
STATE OF WASHINGTON
PROFESSIONAL ENGINEER
No. 46584

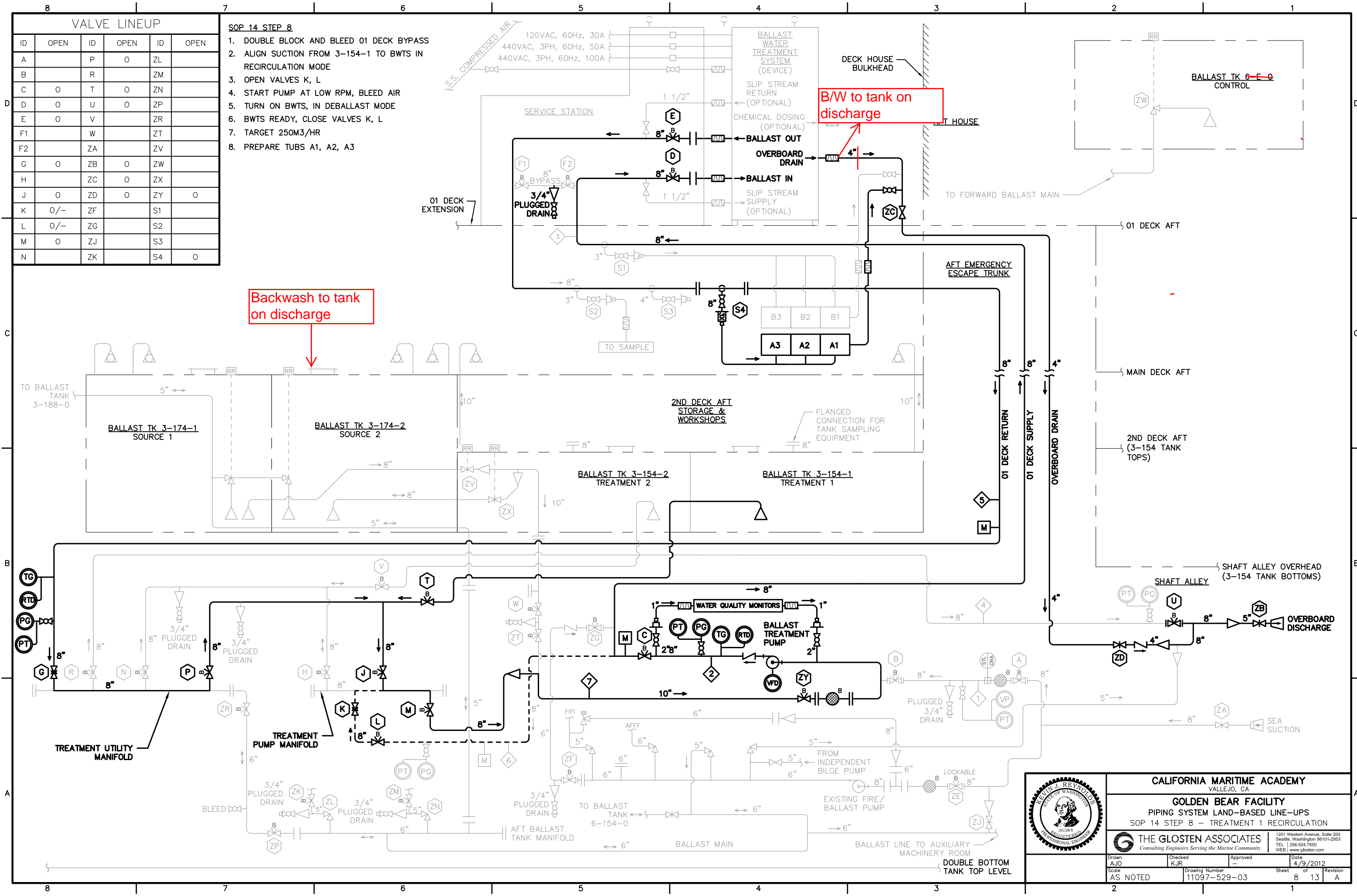
CALIFORNIA MARITIME ACADEMY
VALLEJO, CA

GOLDEN BEAR FACILITY
PIPING SYSTEM LAND-BASED LINE-UPS
SOP 14 STEP 3 - TREATMENT 1 UPTAKE

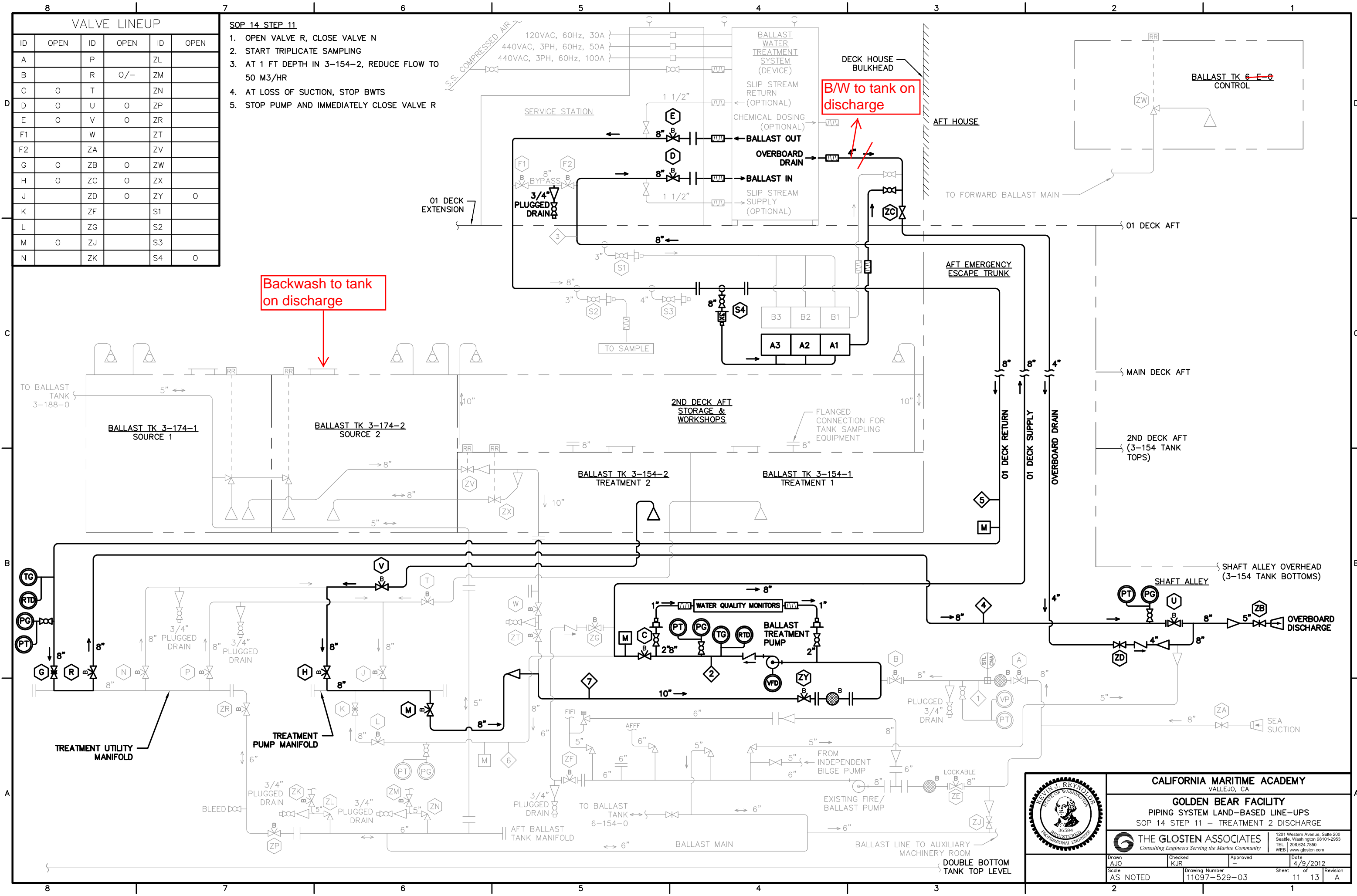
THE GLOSTEN ASSOCIATES
Consulting Engineers Serving the Marine Community

1201 Western Avenue, Suite 200
Seattle, Washington 98101-2953
TEL: 206.624.7850
WEB: www.glosten.com

Drawn AJO	Checked KJR	Approved	Date 4/9/2012
Scale AS NOTED	Drawing Number 11097-529-03	Sheet 4 of 13	Revision A



CALIFORNIA MARITIME ACADEMY VALLEJO, CA			
GOLDEN BEAR FACILITY PIPING SYSTEM LAND-BASED LINE-UPS SOP 14 STEP 8 - TREATMENT 1 RECIRCULATION			
THE GLOSTEN ASSOCIATES Consulting Engineers Serving the Marine Community		1201 Western Avenue, Suite 200 Seattle, Washington 98101-2953 TEL: 206.624.7850 WEB: www.glosten.com	
Drawn AJO	Checked KJR	Approved	Date 4/9/2012
Scale AS NOTED	Drawing Number 11097-529-03	Sheet 8 of 13	Revision A



CALIFORNIA MARITIME ACADEMY VALLEJO, CA			
GOLDEN BEAR FACILITY PIPING SYSTEM LAND-BASED LINE-UPS SOP 14 STEP 11 - TREATMENT 2 DISCHARGE			
THE GLOSTEN ASSOCIATES Consulting Engineers Serving the Marine Community		1201 Western Avenue, Suite 200 Seattle, Washington 98101-2953 TEL: 206.624.7850 WEB: www.glosten.com	
Drawn AJO	Checked KJR	Approved	Date 4/9/2012
Scale AS NOTED	Drawing Number 11097-529-03	Sheet 11 of 13	Revision A

SOP 3 Posted Placards and Piping Line-Ups (SHIPBOARD)

Application	LAND-BASED		SHIPBOARD	XX
-------------	------------	--	-----------	----

(Placard – Post at Pumping Station, Sampling Station, and Laboratory)

Emergency Contacts (listed in notification order)

- William T. Davidson, Facility Director**
Golden Bear Facility
707.654.1304, cell 707.592.4267
bdavidson@csum.edu
- Harry Bolton, Ship Master**
California Maritime Academy
707.654.1192, cell 707.246.7495
HBolton@csum.edu
- Cal Maritime Emergency Operations Center**
707.654.1111
- MLML ES&H Contacts**
Moss Landing Marine Laboratories
831.771.4400
- Jocelyn Douglas, Health and Safety Officer**
831.771.4451, cell 831.750.9563

Additional Project Contacts

Richard S. Muller, Quality Officer
Golden Bear Facility
707.654.1258
rmuller@csum.edu

Veronica Boe, Facilities Program Support
California Maritime Academy
707.654.1156
vboe@csum.edu

Nicholas A. Welschmeyer, PhD, Lead Scientist
Golden Bear Facility
831.771.4439
welschmeyer@mlml.calstate.edu

Jad Mouawad, Senior Engineer
Det Norske Veritas
+47 480 50 902
jad.mouawad@dnv.com

Andrew Daley, Project Manager
Trojan UV
519.457.3400, cell 226.688.7430
adaley@trojanuv.com

Test Cycle Number

PIC Initials

Quality Initials

Date

(Placard – Post at Pumping Station, Sampling Station, and Laboratory)

	Criteria	Uptake Cycles		Discharge Cycles	
		Treatment	Control	Treatment	Control
Treatment Line and Tank					
Average (m ³ /hr)	250 ± 10%				
Total Volume (m ³)	≥ 200				
Control Line and Tank					
Average (m ³ /hr)	250 ± 10%				
Total Volume (m ³)	≥ 200				
Combined Sample Volume (m³)					
Uptake and Control Discharge	≥ 3				
Treatment Discharge	≥ 9				
Ballast Hold Duration (hours)	48+				
Water Quality					
Salinity (PSU)	N/A				
Temperature (Celcius)	N/A				
DOC (mg/L)	N/A				
POC (mg/L)	N/A				
TSS (mg/L)	N/A				
Uptake Living Populations					
≥50 microns (organisms/m ³)	100				
≥10 < 50 microns (organisms/mL)	100				
Control Living Populations					
≥50 microns (organisms/m ³)	10				
≥10 < 50 microns (organisms/mL)	10				

(Placard – Post at Pumping Station, Sampling Station, and Laboratory)

		Treatment Uptake	Control Uptake	Treatment Discharge	Treatment Stripping	Control Discharge	Control Stripping
Pumping Rate	(m3/hr)	250	250	250	50	250	50
Sample Port Location		S-1	S-1	S-4	S-4	S-1	S-1
Pitot Tube I.D.	(inch)	1.61	1.61	3x1.68	3x1.68	1.61	1.61
Sample Tub Set		A	B	A	A	B	B
Sample Rate Average	(gpm)	3x6.64	3x6.64	3x19.8	3x4.00	3x6.64	3x1.33
Sample Volume Target	(m3)	3x1.2	3x1.2	3x3.6	**	3x1.2	**

Flow Rates for 3.6m3 Sample w/ 1.61 Inch I.D. Pitot at 48 Minutes

Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate/ Sample to Manifold (gpm)	4.0	19.1	19.9	20.7
Throttle Flow Meters as per Table				
Tub 1 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 2 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 3 Rate (gpm)*	1.33	6.37	6.64	6.90

Flow Rates for 10.8m3 Sample w/ 3x1.68 Inch I.D. Pitots at 48 Minutes

Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate per Pitot/ Sample to Tanks (gpm)	4.0	19.0	19.8	20.6
Throttle Flow Meters as per Table				
Tub 1 Rate (gpm)*	3.97	19.04	19.83	20.63
Tub 2 Rate (gpm)*	3.97	19.04	19.83	20.63
Tub 3 Rate (gpm)*	3.97	19.04	19.83	20.63

Flow Rates for 3.6m3 Sample w/ 1.61 Inch I.D. Pitot at 48 Minutes

Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate/ Sample to Manifold (gpm)	4.0	19.1	19.9	20.7
Throttle Flow Meters as per Table				
Tub 1 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 2 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 3 Rate (gpm)*	1.33	6.37	6.64	6.90

Test Cycle Number

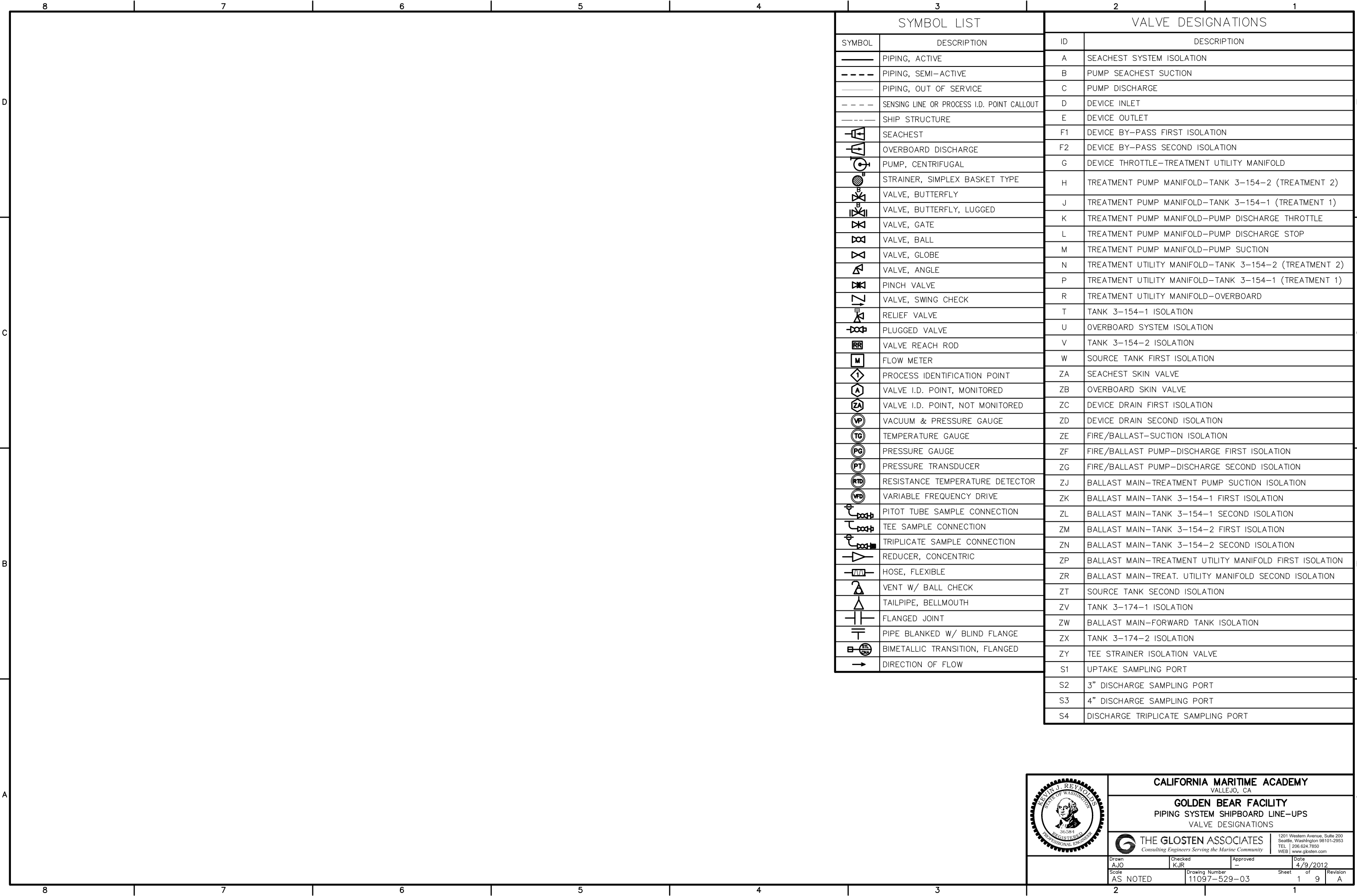
PIC Initials

Quality Initials


Date

SOP 3 (Continued)

The following diagrams provide illustrations of the piping system line-ups.




SYMBOL LIST		VALVE DESIGNATIONS	
SYMBOL	DESCRIPTION	ID	DESCRIPTION
	PIPING, ACTIVE	A	SEACHEST SYSTEM ISOLATION
	PIPING, SEMI-ACTIVE	B	PUMP SEACHEST SUCTION
	PIPING, OUT OF SERVICE	C	PUMP DISCHARGE
	SENSING LINE OR PROCESS I.D. POINT CALLOUT	D	DEVICE INLET
	SHIP STRUCTURE	E	DEVICE OUTLET
	SEACHEST	F1	DEVICE BY-PASS FIRST ISOLATION
	OVERBOARD DISCHARGE	F2	DEVICE BY-PASS SECOND ISOLATION
	PUMP, CENTRIFUGAL	G	DEVICE THROTTLE-TREATMENT UTILITY MANIFOLD
	STRAINER, SIMPLEX BASKET TYPE	H	TREATMENT PUMP MANIFOLD-TANK 3-154-2 (TREATMENT 2)
	VALVE, BUTTERFLY	J	TREATMENT PUMP MANIFOLD-TANK 3-154-1 (TREATMENT 1)
	VALVE, BUTTERFLY, LUGGED	K	TREATMENT PUMP MANIFOLD-PUMP DISCHARGE THROTTLE
	VALVE, GATE	L	TREATMENT PUMP MANIFOLD-PUMP DISCHARGE STOP
	VALVE, BALL	M	TREATMENT PUMP MANIFOLD-PUMP SUCTION
	VALVE, GLOBE	N	TREATMENT UTILITY MANIFOLD-TANK 3-154-2 (TREATMENT 2)
	VALVE, ANGLE	P	TREATMENT UTILITY MANIFOLD-TANK 3-154-1 (TREATMENT 1)
	PINCH VALVE	R	TREATMENT UTILITY MANIFOLD-OVERBOARD
	VALVE, SWING CHECK	T	TANK 3-154-1 ISOLATION
	RELIEF VALVE	U	OVERBOARD SYSTEM ISOLATION
	PLUGGED VALVE	V	TANK 3-154-2 ISOLATION
	VALVE REACH ROD	W	SOURCE TANK FIRST ISOLATION
	FLOW METER	ZA	SEACHEST SKIN VALVE
	PROCESS IDENTIFICATION POINT	ZB	OVERBOARD SKIN VALVE
	VALVE I.D. POINT, MONITORED	ZC	DEVICE DRAIN FIRST ISOLATION
	VALVE I.D. POINT, NOT MONITORED	ZD	DEVICE DRAIN SECOND ISOLATION
	VACUUM & PRESSURE GAUGE	ZE	FIRE/BALLAST-SUCTION ISOLATION
	TEMPERATURE GAUGE	ZF	FIRE/BALLAST PUMP-DISCHARGE FIRST ISOLATION
	PRESSURE GAUGE	ZG	FIRE/BALLAST PUMP-DISCHARGE SECOND ISOLATION
	PRESSURE TRANSDUCER	ZJ	BALLAST MAIN-TREATMENT PUMP SUCTION ISOLATION
	RESISTANCE TEMPERATURE DETECTOR	ZK	BALLAST MAIN-TANK 3-154-1 FIRST ISOLATION
	VARIABLE FREQUENCY DRIVE	ZL	BALLAST MAIN-TANK 3-154-1 SECOND ISOLATION
	PITOT TUBE SAMPLE CONNECTION	ZM	BALLAST MAIN-TANK 3-154-2 FIRST ISOLATION
	TEE SAMPLE CONNECTION	ZN	BALLAST MAIN-TANK 3-154-2 SECOND ISOLATION
	TRIPPLICATE SAMPLE CONNECTION	ZP	BALLAST MAIN-TREATMENT UTILITY MANIFOLD FIRST ISOLATION
	REDUCER, CONCENTRIC	ZR	BALLAST MAIN-TREAT. UTILITY MANIFOLD SECOND ISOLATION
	HOSE, FLEXIBLE	ZT	SOURCE TANK SECOND ISOLATION
	VENT W/ BALL CHECK	ZV	TANK 3-174-1 ISOLATION
	TAILPIPE, BELLMOUTH	ZW	BALLAST MAIN-FORWARD TANK ISOLATION
	FLANGED JOINT	ZX	TANK 3-174-2 ISOLATION
	PIPE BLANKED W/ BLIND FLANGE	ZY	TEE STRAINER ISOLATION VALVE
	BIMETALLIC TRANSITION, FLANGED	S1	UPTAKE SAMPLING PORT
	DIRECTION OF FLOW	S2	3" DISCHARGE SAMPLING PORT
		S3	4" DISCHARGE SAMPLING PORT
		S4	DISCHARGE TRIPPLICATE SAMPLING PORT



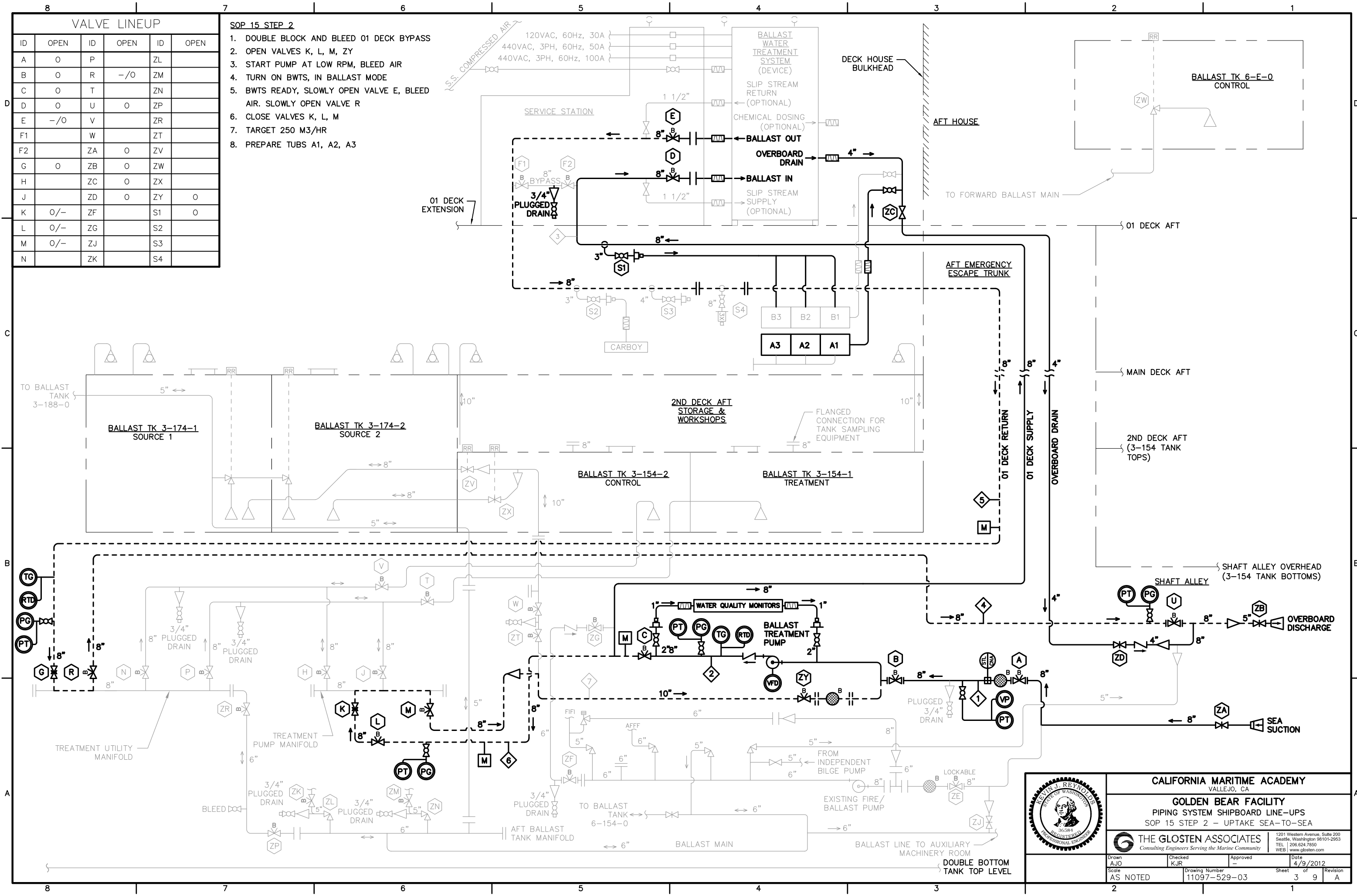
CALIFORNIA MARITIME ACADEMY
VALLEJO, CA

GOLDEN BEAR FACILITY
PIPING SYSTEM SHIPBOARD LINE-UPS
VALVE DESIGNATIONS

 **THE GLOSTEN ASSOCIATES**
Consulting Engineers Serving the Marine Community

1201 Western Avenue, Suite 200
Seattle, Washington 98101-2953
TEL | 206.624.7850
WEB | www.glosten.com

Drawn AJO	Checked KJR	Approved	Date 4/9/2012
Scale AS NOTED	Drawing Number 11097-529-03	Sheet 1 of 9	Revision A



CALIFORNIA MARITIME ACADEMY VALLEJO, CA			
GOLDEN BEAR FACILITY PIPING SYSTEM SHIPBOARD LINE-UPS SOP 15 STEP 2 - UPTAKE SEA-TO-SEA			
THE GLOSTEN ASSOCIATES Consulting Engineers Serving the Marine Community		1201 Western Avenue, Suite 200 Seattle, Washington 98101-2953 TEL 206.624.7850 WEB www.glosten.com	
Drawn AJO	Checked KJR	Approved	Date 4/9/2012
Scale AS NOTED	Drawing Number 11097-529-03	Sheet 3	of 9 Revision A

SOP 4 BWTS Commissioning Data Sheets

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

Manufacture's Authorized Representative

The BWTS has been installed and commissioned in accordance to manufacturer's requirements.

Name

Title

Date

SOP 4, Step 1 Arrival Inspection

This step provides the procedure for inspection of the BWTS on arrival to the facility for damage and readiness for installation by PIC, Quality and Installation Contractor. All items checked as “no” shall be addressed in “Notes” or in space provided.

Rigging and Installation Contractor: _____.

_____ Print	_____ Sign	_____ Date
----------------	---------------	---------------

INITIAL INSPECTION. Initial following completion _____.

Damage? yes__ no__
 ISO rack suitable? yes__ no__
 Interference and protrusions? yes__ no__
 Weight acceptable? yes__ no__

CONNECTIONS AND INTERFACE SURVEY. Initial following completion _____.

Standard 8-inch flange in/out? yes__ no__
 Arrangement ok? yes__ no__
 4" drain connection? yes__ no__
 Air/water requirements met? yes__ no__
 Adequate electrical cabling? yes__ no__ List requirements in Notes.
 Electrical power requirements met? yes__ no__
 User interface cabling requirements? yes__ no__
 BWTS data connection functional? yes__ no__
 Other connections required? yes__ no__ Detail in Notes. _____.

Notes: _____

SOP 4, Step 2 Installation

This step provides the procedure for installation of the BWTS. All items checked as “no” shall be addressed in “Notes” or in space provided.

Initial following completion _____.

- Container secured to rigid foundations on the vessel yes__ no__
- 8" Ballast Inlet connection mated to shipboard piping yes__ no__
- 8" Ballast Outlet connection mated to shipboard piping yes__ no__
- 4" Backflush Drain connection mated to shipboard piping yes__ no__
- 1/2" Compressed Air inlet connection mated to shipboard piping yes__ no__
- 100 amp electrical cables connected to lugs at 3 phase disconnect switch and ship service distribution panel yes__ no__
- Data interface cables connected between BWTS and IMAC yes__ no__

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

SOP 4, Step 3 Service

This step provides the procedure for inspection and servicing of the BWTS prior to start-up. All items checked as “no” shall be addressed in “Notes” or in space provided.

Initial following completion _____.

- UV lamps inspected, no broken or damaged lamps found yes__ no__
- Filter assemblies inspected yes__ no__
- Control interface functions normally yes__ no__

Notes: _____

SOP 4, Step 4 Operational Check

This step provides the procedure for pre-operational and functional checks of the BWTS. All items checked as “no” shall be addressed in “Notes” or in space provided.

Note: A Shake Down test of the BWTS is part of a separate detailed procedure and data log.

Pre-Operational Checks

Initial following completion _____.

- All BWTS piping, filters housings, UV assembly are flooded with seawater and high points vented so that no trapped air remains yes__ no__
- Pneumatic pressure regulated to the correct pressure at the BWTS inlet connection yes__ no__
- All pneumatically actuated valves cycled via manual override of solenoids in accordance with the BWTS Technical Bulletin yes__ no__
- All circuit interruption devices closed and correct electrical voltage is present at the BWTS control cabinet yes__ no__

System Start-Up and Operational Checks “Ballast Mode”

Initial following completion _____.

- System valves lined up for for **sea-to-sea** mode with BWTS bypass shut yes__ no__
- Ballast treatment pump is turned on and air bled from piping system prior to turning BWTS on yes__ no__
- BWTS is started in accordance with the BWTS Technical Bulletin using “ballast” mode yes__ no__
- Upon commencement of “ballast” mode of operation the design flow rate of 250 cu m/hr through the BWTS (both filter and UV assemblies) is verified at point 5 flow meter yes__ no__
- UV lamp testing and sensor verification carried out in accordance with the BWTS Technical Bulletin yes__ no__
- Proper functioning of the filter system backflush cycle is observed yes__ no__
- BWTS data download matches operations yes__ no__
- System shut down is carried out in accordance with the BWTS Technical Bulletin yes__ no__
- Ballast Treatment Pump is secured after completion of the shut-down and filter backflush yes__ no__

System Start-Up and Operational Checks “De-Ballast Mode”

Initial following completion _____.

- System valves lined up for **sea-to-sea** mode yes__ no__
- At least 10 minutes have elapsed since BWTS “ballast” mode shut-down yes__ no__
- Ballast treatment pump is turned on and air bled from piping system prior to turning BWTS on yes__ no__
- BWTS is started in accordance with the BWTS Technical Bulletin using “de-ballast” mode yes__ no__
- Upon commencement of “de-ballast” mode of operation the design flow rate of 250 cu m/hr through the BWTS (UV assembly only) verified at point 5 flow meter yes__ no__
- UV lamp testing and sensor verification carried out in accordance with the BWTS Technical Bulletin yes__ no__
- BWTS data download matches operations yes__ no__
- System shut down is carried out in accordance with the BWTS Technical Bulletin yes__ no__

Notes: _____

SOP 4, Step 5 Lay-Up

This step provides the procedure for lay-up of the BWTS after completion of testing. All items checked as “no” shall be addressed in “Notes” or in space provided. The system automatically drains when shutdown, leaving it in a “dry lay-up” condition between tests.

Draining Procedure

Initial following completion _____.

- BWTS has been secured from operation and treatment pump is shut off yes__ no__
- Device automatically drains upon shutdown yes__ no__

Flushing and Draining Procedure (for extended lay-up and prior to shipping)

Initial following completion _____.

- Temporary hose connection is made between nearest fresh water hose bibb and plugged drain between valves F1 and F2 yes__ no__
- Sample pitot port S2 or S3 is open, temporary hose is fitted and routed overboard yes__ no__
- Hose bibb valve is opened supplying pressurized fresh water to the BWTS inlet yes__ no__
- Control valve solenoids are manually actuated such that the fresh water is allowed to flow through the filter and UV assemblies yes__ no__
- Fresh water flushing is run for five minutes yes__ no__
- Fresh water is secured, hoses are disconnected, sample pitot valve returned to the closed position yes__ no__
- High point vents on filter and UV assemblies are opened, fresh water drained off via the drain valve left open and various BWTS drains yes__ no__
- Upon completion of draining, all vents and drain valves are closed, device inlet and outlet valves are closed yes__ no__
- Circuit breaker providing electrical power to the BWTS is opened yes__ no__

Notes: _____

SOP 5 BWTS Shakedown Test Data Sheets

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Test Cycle Piping Diagrams identify system process points, instrumentation locations, and designate valve names. They are available in the automation system, on Facility laminated placards, and in key documents.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and Online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

The function of the Shakedown testing is to provide adequate run time and run conditions such that most mechanical problems with the installed BWTS will become obvious, prior to commencement of biological efficacy testing. The manufacturer’s representative is expected to be on-site to make any needed repairs during this time.

SOP 5, Step 1 Sea-to-Sea Mode (see Test Cycle Diagram)

This step circulates seawater from the seachest to the overboard, circulating the seawater past the 01 Deck.

- Align Valves and Bleeds as per Diagram. Throttle Valves G at Two (2) Threads Showing.
- Place BWTS to Standby. Bypass valves should be shut.
- Start Facility Pump at 650 RPM. Bleed any air from high point vents on 01 deck.
- Place BWTS in Ballast Mode. After BWTS warm-up period, use Valve G and Pump Speed, Adjust to ~250 m3/hr and 240 kPa at Process Points #5. Run for two (2) hours.
- Log System Data.
- Use Notes to Log BWTS monitored parameters.

Sea-to-Sea Mode Logs

SYSTEM PARAMETERS			TIME Use 24 HR (####)		
Position	#1	#2	#4	#5	#6
Flow (m3/hr)	<div></div>		<div></div>		<div></div>
Temp (C)	<div></div>		<div></div>		<div></div>
Press (kPa)	<div></div>		<div></div>		<div></div>

Notes:

SOP 5, Step 2 Stressing Cycles

This step starts and stops the BWTS and Facility Pump, as well as increases flow rates and pressures beyond what is expected during the Biological Efficacy test cycle.

- Maintain sea-to-sea mode.
- Stop and start facility pump three (3) times. Log BWTS response in notes.
- With facility pump running, stop and start BWTS three (3) times shifting between Ballast and De-Ballast modes. Log BWTS response in notes.
- Leave BWTS off and secure facility pump. Leave off for ~12 hours (overnight).
- Shift to facility piping to recirculation mode. Restart pump and then BWTS.
- Increase pump speed to maintain 10% above planned testing flow rate to ~275 m³/hr at position 5 flowmeter. Throttle Valve G until Process Point 5 reaches ~300 kPa. Run for four (4) hours. Log system data. Secure BWTS and facility piping system.

Stressing Cycles Log

SYSTEM PARAMETERS			TIME Use 24 HR (#####)		
Position	#1	#2	#4	#5	#6
Flow (m ³ /hr)					
Temp (C)					
Press (kPa)					

SYSTEM PARAMETERS			TIME Use 24 HR (#####)		
Position	#1	#2	#4	#5	#6
Flow (m ³ /hr)					
Temp (C)					
Press (kPa)					

Notes:

SOP 6 Quality Control Checklist for Cycle (LAND-BASED)

Application	LAND-BASED	XX	SHIPBOARD	
-------------	------------	----	-----------	--

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as it is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge:

Quality Officer:

Sign _____ Date _____

Sign _____ Date _____

Cycle _____

Event _____ Event _____

Event _____ Event _____

Event _____ Event _____

Event _____ Event _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

SOP 6, Step 1 Documentation Verification

Initial each completed item.

- Verify that Test Placards are at Pumping Station, Sampling Station, and Laboratory
 - Emergency Contact Numbers _____.
 - Valid Test Parameters _____.
 - Sample and Pumping Rate Coordination _____.
 - Sampling Rates _____.
- Distribute and track SOP document completion in accordance with below checklist.
 - Identify approved versions of protocols and data logs _____.
 - Determine “hand log” or “online log” for each data log _____.

*Note: use Documentation Verification Table in Step 4 to track completion and record of all documents.

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Documentation Verification Table

Document Information		Completion Log				Notes/File Name
Name	Date (MM/DD)	Initial	Hand	Online		
Standard Operating Procedures						
SOP 1 Quality Control Checklist for Project						
SOP 2 Posted Placards and Piping Line-Ups (Land-Based)						
SOP 4 BWTS Commissioning						
SOP 5 BWTS Shakedown Test						
SOP 6 Quality Control Checklist for Cycle (Land-Based)						
SOP 8 Tank Cleaning (Land-Based)						
SOP 10 Preparation of Source Tanks (Land-Based)						
SOP 11 Calibration of Sampling System Flow Meters						
SOP 12 Calibration of Sea-Bird Thermosalinograph (TSG)						
SOP 13 Calibration of Flow Cytometer & Lab Fluorometer						
SOP 14 Operation of BWTS and Facility Piping System (Land-Based)						
SOP 16 Operation of Ballast Water Sampling System (Land-Based)						
SOP 18 Chain of Custody Record						
SOP 19 Biological Analysis Data Sheets						
SOP 20 Poke and Probe Viability Determination for Organisms ≥50 μm						
SOP 21 Most Probable Number (MPN) Determination of Viable Phytoplankton Cells ≥10 to <50 μm, Chlorophyll-based						
SOP 22 C-14 Primary Production						
SOP 23 Chlorophyll <i>a</i>						
SOP 24 Heterotrophic Bacteria Plate Counts for Organisms <10 μm						
SOP 25 Indicator Microbes <i>E. coli</i> and <i>Enterococci</i> for Organisms <10 μm						
SOP 26 Indicator Microbes <i>Vibrio Cholerae</i> Serotype 01 and <i>Vibrio Cholerae</i> Serotype 0139 for Organisms <10 μm						
SOP 27 FDA-Based, Flow Cytometric Analysis of Viable Organisms ≥10 μm but <50 μm						
SOP 28 FDA/CMFDA Epifluorescence Analysis of Viable Organisms ≥10 μm but <50 μm						
SOP 29 In-situ Thermosalinograph (TSG) Operation, Data Collection, and Data Processing						

Test Cycle Number

PIC Initials

Quality Initials

Date _____

[illegible]

Test Cycle Number _____

PIC Initials _____

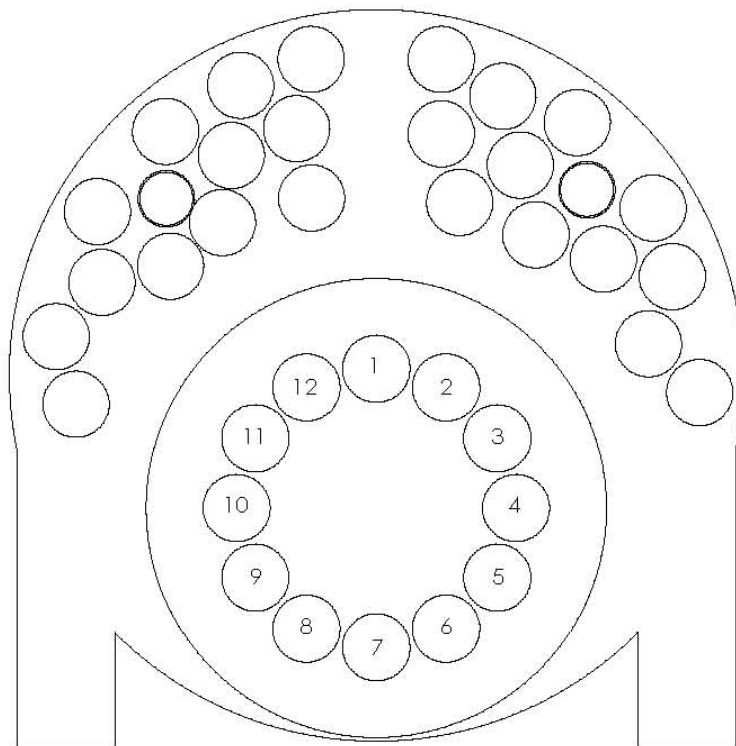
Quality Initials _____

Date _____

SOP 6, Step 2 BWTS Pre-Operation Checklist

Initial following completion _____.

Filter Element Model installed _____.



FILTER NUMBERING SCHEME

1.	2.
3.	4.
5.	6.
7.	8.
9.	10.
11.	12.

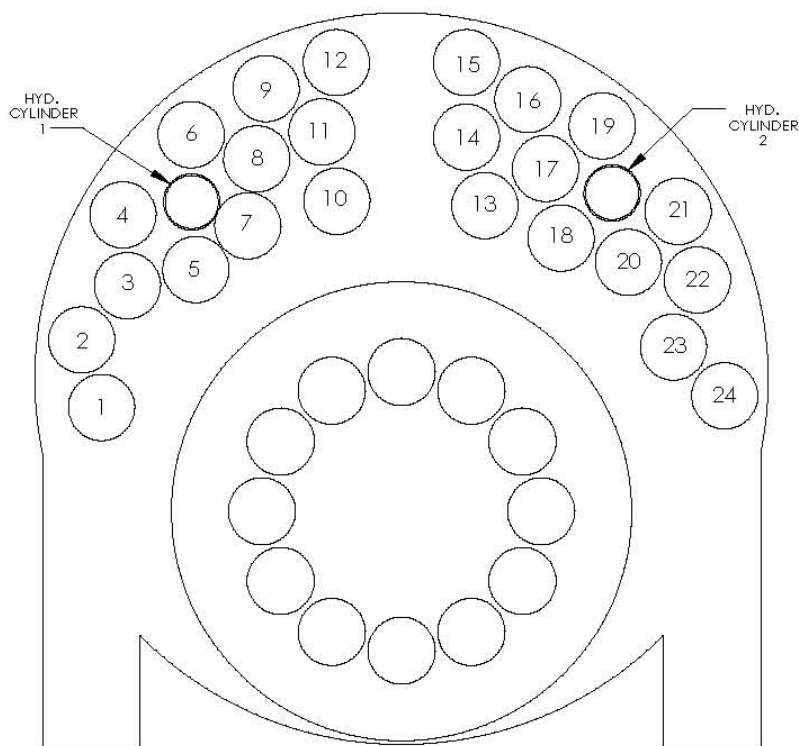
Test Cycle Number _____

PIC Initials _____

Quality Initials _____

Date _____

UV Lamp Model installed _____.



LAMP NUMBERING SCHEME

1.	2.
3.	4.
5.	6.
7.	8.
9.	10.
11.	12.
13.	14.
15.	16.
17.	18.
19.	20.
21.	22.
23.	24.

Backflush Initiation Differential Pressure setting _____.

SOP 6, Step 3 Test Cycle Automation Initiation, Tracking, and Error Logging

Initial each completed item.

- Initiate Automation System Test Cycle Tracking _____.
- Verify Parameter Display of Three (3) (Randomly Selected) Field Devices _____.
- Document Errors in Notes _____.

Field Device	Parameter Display			Verification
Name	At Field Device	On UI Screen	In Report	Date/Time

Notes: _____

SOP 6, Step 4 Validating Test Parameters

Validate Test Parameters in below log. Report the results to Director at conclusion of Uptake Events and Discharge Events.

	Criteria		Uptake Events			Discharge Events		
			Treat 1	Treat 2	Control	Treat 1	Treat 2	Control
Treatment Line and Tanks								
Average (m³/hr)	250 ± 10%							
Volume at end Cycle (m³)	200 (-0%/+10%)							
Control Line and Tank								
Total Volume (m³)	200 (-0%/+10%)							
Combined Sample Volume (m³)								
Uptake	≥ 1							
Control Discharge	≥ 3							
Treatment Discharge	≥ 9							
Ballast Hold Duration (hours)	120 (-0%/+10%)							
Water Quality	Brackish	Salt						
Salinity (PSU)	10 - 20	>32						
Temperature (Celcius)	4 - 35	4 - 35						
DOC (mg/L)	≥ 6	≥ 6						
POC (mg/L)	≥ 5	≥ 4						
TSS (mg/L)	≥ 50	≥ 24						
Uptake Living Populations								
≥50 microns (organisms/m³)	10^5							
≥10 < 50 microns (organisms/mL)	10^3							
<10 microns (bacteria/mL)	10^4							
Control Living Populations								
≥50 microns (organisms/m³)	100							
≥10 < 50 microns (organisms/mL)	100							
<10 microns (bacteria/mL)	500							

Notes: _____

SOP 6, Step 5 Data Tracking, Filing, and Recording

Hand SOP Documents

- Collect all SOPs at the end of each Test Cycle and complete Documentation Verification Table (Step 1).
- Scan documents into online information system, providing unique name. For example, for Test Cycle A 12-3-14 the SOP 12 would be: "A_12-03-14_SOP12". Scan the entire document, including pages that have data.
- Save and note file name in Documentation Verification Table.

Hand Non-SOP Documents

- Collect all other hand documents at the end of each Test Cycle and record into the Documentation Verification Table. Examples include science notebooks, test variation reports, and document redlines.
- Scan documents into online information system, providing a unique name.
- Save and note file name in Documentation Verification Table.

Online Logs, Checklists, Reports.

- Identify and record all online data files at the end of each Test Cycle into the Documentation Verification Table.
- Copy document into online information system, providing unique name using same convention as for hand data (above).
- Save and note file name in Documentation Verification Table.

Notes: _____

SOP 6, Step 6 Verification Data Record

Produce Read-Only DVDs for each completed Test Cycle as Follows:

- Download to Secure Directory.
 - Automation System Database
 - Hand Data Scans
 - Online Data (In-situ monitors and probes)
- DVD Production.
 - Burn Two (2) DVDs.
 - Scribe each DVD: Test Cycle Name and Date. For example, a DVD for test day March 14th 2012, might read: “Test Cycle A - 12-03-14-1332, Data Recorded 12-03-14”
- DVD Storage.
 - One (1) on-site in Chief Engineer office maintained by Facility Director.
 - One (1) off-site at Simulation Center maintained by Quality Officer and Facility Director.

Notes: _____

SOP 7 Quality Control Checklist for Cycle (SHIPBOARD)

Application	LAND-BASED		SHIPBOARD	XX
-------------	------------	--	-----------	----

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as it is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge:

Quality Officer:

Sign _____ Date _____

Sign _____ Date _____

Cycle _____

Event _____

Event _____

Event _____

Event _____

Event _____

Event _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

_____	_____	_____	_____
Test Cycle Number	PIC Initials	Quality Initials	Date

SOP 7, Step 1 Documentation Verification

Initial each completed item.

- Verify that Test Placards are at Pumping Station, Sampling Station, and Laboratory
 - Emergency Contact Numbers _____.
 - Valid Test Parameters _____.
 - Sample and Pumping Rate Coordination _____.
 - Sampling Rates _____.
- Distribute and track SOP document completion in accordance with below checklist.
 - Identify approved versions of protocols and data logs _____.
 - Determine “hand log” or “online log” for each data log _____.

*Note: use Documentation Verification Table in Step 4 to track completion and record of all documents.

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Documentation Verification Table

Document Information		Completion Log				
Name	Date (MM/DD)	Initial	Hand	Online	Notes/File Name	
Standard Operating Procedures						
SOP 1 Quality Control Checklist for Project						
SOP 3 Posted Placards and Piping Line-Ups (Shipboard)						
SOP 4 BWTS Commissioning						
SOP 5 BWTS Shakedown Test						
SOP 7 Quality Control Checklist for Cycle (Shipboard)						
SOP 9 Tank Cleaning (Shipboard)						
SOP 11 Calibration of Sampling System Flow Meters						
SOP 12 Calibration of Sea-Bird Thermosalinograph (TSG)						
SOP 13 Calibration of Flow Cytometer & Lab Fluorometer						
SOP 15 Operation of BWTS and Facility Piping System (Shipboard)						
SOP 17 Operation of Ballast Water Sampling System (Shipboard)						
SOP 18 Chain of Custody Record						
SOP 19 Biological Analysis Data Sheets						
SOP 20 Poke and Probe Viability Determination for Organisms ≥50 μm						
SOP 21 Most Probable Number (MPN) Determination of Viable Phytoplankton Cells ≥10 to <50 μm, Chlorophyll-based						
SOP 22 C-14 Primary Production						
SOP 23 Chlorophyll <i>a</i>						
SOP 24 Heterotrophic Bacteria Plate Counts for Organisms <10 μm						
SOP 25 Indicator Microbes <i>E. coli</i> and <i>Enterococci</i> for Organisms <10 μm						
SOP 26 Indicator Microbes <i>Vibrio Cholerae</i> Serotype 01 and <i>Vibrio Cholerae</i> Serotype 0139 for Organisms <10 μm						
SOP 27 FDA-Based, Flow Cytometric Analysis of Viable Organisms ≥10 μm but <50 μm						
SOP 28 FDA/CMFDA Epifluorescence Analysis of Viable Organisms ≥10 μm but <50 μm						
SOP 29 In-situ Thermosalinograph (TSG) Operation, Data Collection, and Data Processing						

Test Cycle Number

PIC Initials

Quality Initials

Date _____

[illegible]

Test Cycle Number _____

PIC Initials _____

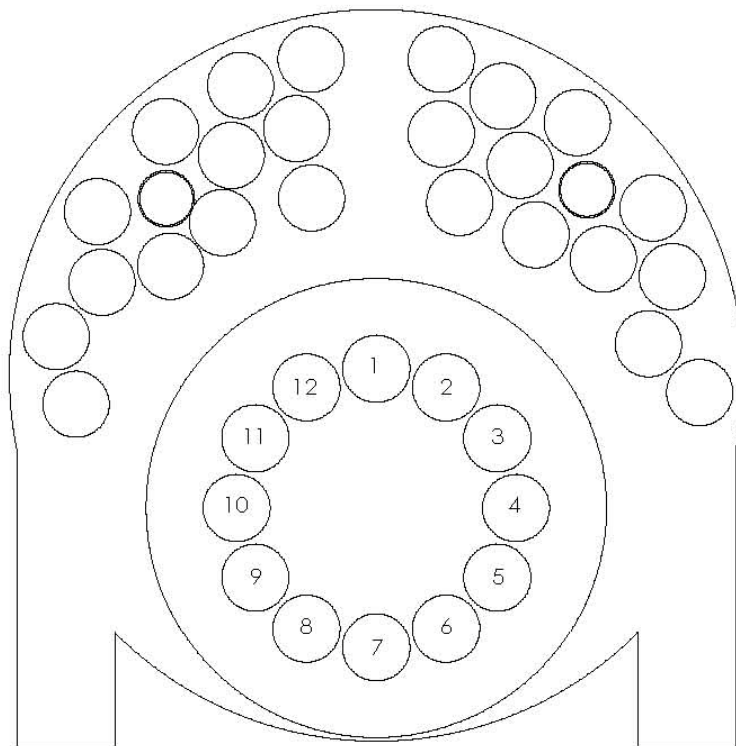
Quality Initials _____

Date _____

SOP 7, Step 2 BWTS Pre-Operation Checklist

Initial following completion _____.

Filter Element Model installed _____.



FILTER NUMBERING SCHEME

1.	2.
3.	4.
5.	6.
7.	8.
9.	10.
11.	12.

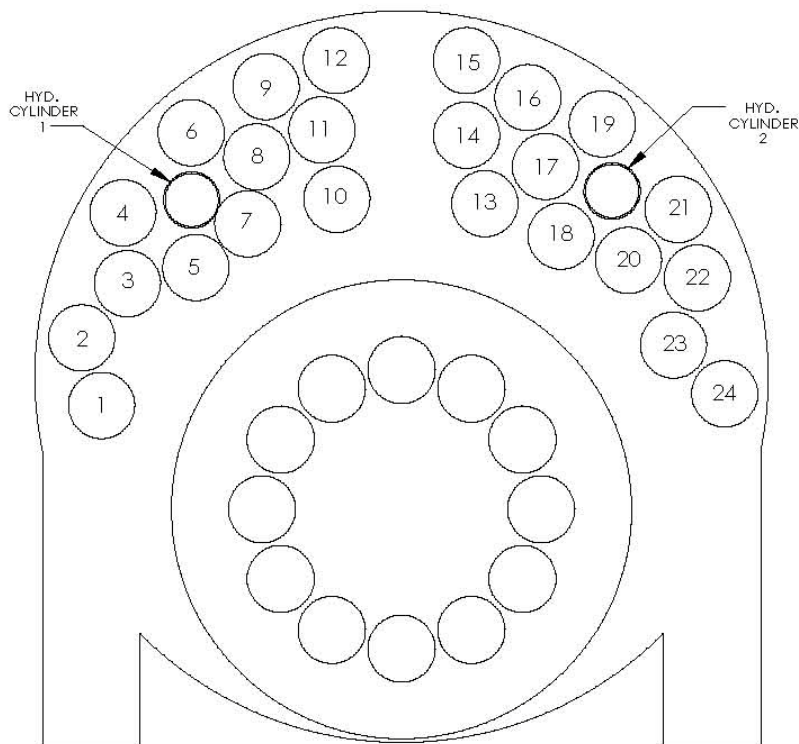
Test Cycle Number _____

PIC Initials _____

Quality Initials _____

Date _____

UV Lamp Model installed _____.



LAMP NUMBERING SCHEME

1.	2.
3.	4.
5.	6.
7.	8.
9.	10.
11.	12.
13.	14.
15.	16.
17.	18.
19.	20.
21.	22.
23.	24.

Backflush Initiation Differential Pressure setting _____.

Test Cycle Number

PIC Initials

Quality Initials

Date

SOP 7, Step 3 Test Cycle Automation Initiation, Tracking, and Error Logging

Initial each completed item.

- Initiate Automation System Test Cycle Tracking _____.
- Verify Parameter Display of Three (3) (Randomly Selected) Field Devices _____.
- Document Errors in Notes _____.

Field Device	Parameter Display			Verification
Name	At Field Device	On UI Screen	In Report	Date/Time

Notes: _____

SOP 7, Step 4 Validating Test Parameters

Validate Test Parameters in below log. Report the results to Director at conclusion of Uptake Events and Discharge Events.

	Criteria	Uptake Cycles		Discharge Cycles	
		Treatment	Control	Treatment	Control
Treatment Line and Tank					
Average (m ³ /hr)	250 ± 10%				
Total Volume (m ³)	≥ 200				
Control Line and Tank					
Average (m ³ /hr)	250 ± 10%				
Total Volume (m ³)	≥ 200				
Combined Sample Volume (m³)					
Uptake and Control Discharge	≥ 3				
Treatment Discharge	≥ 9				
Ballast Hold Duration (hours)	48+				
Water Quality					
Salinity (PSU)	N/A				
Temperature (Celcius)	N/A				
DOC (mg/L)	N/A				
POC (mg/L)	N/A				
TSS (mg/L)	N/A				
Uptake Living Populations					
≥50 microns (organisms/m ³)	100				
≥10 < 50 microns (organisms/mL)	100				
Control Living Populations					
≥50 microns (organisms/m ³)	10				
≥10 < 50 microns (organisms/mL)	10				

Notes:

SOP 7, Step 5 Data Tracking, Filing, and Recording

Hand SOP Documents

- Collect all SOPs at the end of each Test Cycle and complete Documentation Verification Table (Step 1).
- Scan documents into online information system, providing unique name. For example, for Test Cycle A 12-3-14 the SOP 12 would be: "A_12-03-14_SOP12". Scan the entire document, including pages that have data.
- Save and note file name in Documentation Verification Table.

Hand Non-SOP Documents

- Collect all other hand documents at the end of each Test Cycle and record into the Documentation Verification Table. Examples include science notebooks, test variation reports, and document redlines.
- Scan documents into online information system, providing a unique name.
- Save and note file name in Documentation Verification Table.

Online Logs, Checklists, Reports.

- Identify and record all online data files at the end of each Test Cycle into the Documentation Verification Table.
- Copy document into online information system, providing unique name using same convention as for hand data (above).
- Save and note file name in Documentation Verification Table.

Notes: _____

SOP 7, Step 6 Verification Data Record

Produce Read-Only DVDs for each completed Test Cycle as Follows:

- Download to Secure Directory.
 - Automation System Database
 - Hand Data Scans
 - Online Data (In-situ monitors and probes)
- DVD Production.
 - Burn Two (2) DVDs.
 - Scribe each DVD: Test Cycle Name and Date. For example, a DVD for test day March 14th 2012, might read: “Test Cycle A - 12-03-14-1332, Data Recorded 12-03-14”
- DVD Storage.
 - One (1) on-site in Chief Engineer office maintained by Facility Director.
 - One (1) off-site at Simulation Center maintained by Quality Officer and Facility Director.

Notes: _____

SOP 8 Tank Cleaning (LAND-BASED)

Application	LAND-BASED	XX	SHIPBOARD	
-------------	------------	----	-----------	--

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and Online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

SOP 8, Step 1 Overview

These instructions provide the procedures for certifying safe for entry, inspecting, rinsing (including removal of sediment), and disinfecting Facility tanks. These tanks include:

- Source Tanks 3-174-1 and 3-174-2. These two tanks are to be inspected only prior to each test cycle to document the level of sediment in the tanks. Neither rinsing nor disinfection of these tanks is required.
- Control Tank 6-E-0. This single tank is to be inspected and rinsed. Disinfection of this tank is not required.
- Treatment Tanks 3-154-1 and 3-154-2. These two tanks are to be inspected, rinsed, and disinfected prior to each test cycle.

Track Procedure Steps with the following Table.

5-104-1&2 Control

Procedure Steps	Source Tanks		Control	Test Tanks	
	3-174-1	3-174-2	6-E-0	3-154-1	3-154-2
Safe for Entry of Tank(s)					
Ventilation Start (Date/Time)					
Certified Safe for Entry (Initial)					
Ventilation Stop (Date/Time)					
Inspecting Tank(s)					
Special Notes (Note #)					
Sediment					
Deepest (mm)					
Average Depth (mm)					
Tank Bottom Coverage (%)					
Rinsing Tank(s)					
Equipment Prepared (initial)					
Silt Mucked (initial)					
Chlorine Sprayed (initial)					
Fresh Water Rinse (initial)					
Water Removed (initial)					
No Chlorine Residual (initial)					

Notes: _____

SOP 8, Step 2 Certifying Tank(s) “Safe for Entry”

This step provides the procedure for ventilating, testing, and certifying a tank for being safe for entry for inspection and cleaning purposes. This step must be completed for ALL facility tanks. Use the SOP log sheet to track progress.

Preparation.

- PIC (or contractor) reviews ship tank entry procedures.
- PIC (or contractor) trains task personnel.
- PIC (or contractor) engages certified marine chemist.

Ventilation.

- Manually sound tank to confirm it is empty.
- Open access hatches.
- Install safety rails.
- Install portable ventilator to tank vent terminus and vent for 12 hours minimum.
- Ventilate continually through entire tank cleaning operation:

Certification. Engage marine chemist to inspect and certify EACH tank as safe for entry. Post notices at each tank entry.

SOP 8, Step 3 Inspecting Tank(s)

This step provides the procedure for inspecting the condition of a tank, with special attention to sediment loads. Use the SOP log sheet to log data.

- General inspection of tank condition. Make notes as needed.
- Measurement of sediment in tank. Note deepest sediment load in millimeters. Estimate average sediment load in millimeters. Estimate percentage of tank bottom covered with sediment.

SOP 8, Step 4 Rinsing Tank(s) without Disinfection

This step provides the procedure for rinsing a tank and removing accumulated sediment WITHOUT disinfection.

Preparation.

- PIC Reviews Tank Cleaning Procedures with contractor lead.
- Contractor supplied hoses and pressure washers are connected to fresh water supply.
- Portable pumps are staged in tank.

Rinse.

- Muck any collected silt from tank using buckets as needed.
- Thoroughly rinse all tank surfaces with generous amounts of fresh water.
- Tank is continuously pumped empty ensuring no standing water in tank bottom or piping.

Inspection.

- Tank is inspected with contractor to confirm all silt and debris is removed from tank frames and surfaces. Tank is free of collected water.
- **Any collected** solids are disposed accordance with all state and federal regulations.

SOP 8, Step 5 Rinsing Tank(s) with Disinfection

This step provides the procedure for rinsing a tank and removing accumulated sediment WITH disinfection.

Preparation.

- PIC Reviews Tank Cleaning Procedures with contractor lead.
- Contractor supplied hoses and pressure washers are connected to fresh water supply.
- Portable pumps are staged in tank.
- Train and outfit all tank cleaning personnel with PPE appropriate to working with 200ppm chlorine solution in confined space.
- Outfit personnel with ability to meter chlorine solution at 200ppm (approximately 1 TBSP of 5% laundry bleach solution per gallon of fresh water) into pressure washers.

Rinse and disinfect.

- Muck any collected silt from tank using buckets as needed.
- Spray chlorine solution to contact ALL horizontal and vertical surfaces of tank. Ladders shall be utilized to reach high and inaccessible places. Particular attention shall be paid to hull framing and tank top stiffeners and any other “hard to reach” locations.
- After a short contact time (not less than 5 minutes, but no more than the time it takes to spray down tank), thoroughly rinse all tank surfaces with generous amounts of fresh water.
- Thoroughly rinse all tank surfaces with generous amounts of fresh water.
- Tank is continuously pumped empty ensuring no standing water in tank bottom or piping.

Inspection.

- Tank is inspected with contractor to confirm all silt and debris is removed from tank frames and surfaces. Tank is free of collected water.
- Using “chlorine test strips” to determine residual chlorine at least six (6) random spot checks of wet surfaces do not exceed 3ppm.
- **Any collected** solids are disposed accordance with all state and federal regulations.

SOP 9 Tank Cleaning (SHIPBOARD)

Application	LAND-BASED		SHIPBOARD	XX
-------------	------------	--	-----------	----

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and Online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

*****Shipboard tank cleaning only occurs prior to first Shipboard test. Tanks are NOT cleaned between each subsequent Shipboard test*****

SOP 9, Step 1 Overview

These instructions provide the procedures for certifying safe for entry, inspecting, rinsing (including removal of sediment), and disinfecting Facility tanks. These tanks include:

- Treatment Tank 3-154-1. This tank is to be inspected, rinsed, and disinfected prior to Shipboard testing.
- Control Tank 3-154-2. This single tank is to be inspected and rinsed prior to Shipboard testing. This tank is not disinfected.

Track Procedure Steps with the following Table.

Procedure Steps	Treatment	Control
	3-154-1	3-154-2
Safe for Entry of Tank(s)		
Ventilation Start (Date/Time)		
Certified Safe for Entry (Initial)		
Ventilation Stop (Date/Time)		
Inspecting Tank(s)		
Special Notes (Note #)		
Sediment		
Deepest (mm)		
Average Depth (mm)		
Tank Bottom Coverage (%)		
Rinsing Tank(s)		
Equipment Prepared (initial)		
Silt Mucked (initial)		
Chlorine Sprayed (initial)		
Fresh Water Rinse (initial)		
Water Removed (initial)		
No Chlorine Residual (initial)		

Notes: _____

SOP 9, Step 2 Certifying Tanks “Safe for Entry”

This step provides the procedure for ventilating, testing, and certifying a tank for being safe for entry for inspection and cleaning purposes. This step must be completed for both facility tanks. Use the SOP log sheet to track progress.

Preparation.

- PIC (or contractor) reviews ship tank entry procedures.
- PIC (or contractor) trains task personnel.
- PIC (or contractor) engages certified marine chemist.

Ventilation.

- Manually sound tank to confirm it is empty.
- Open access hatches.
- Install safety rails.
- Install portable ventilator to tank vent terminus and vent for 12 hours minimum.
- Ventilate continually through entire tank cleaning operation:

Certification. Engage marine chemist to inspect and certify EACH tank as safe for entry. Post notices at each tank entry.

SOP 9, Step 3 Inspecting Tanks

This step provides the procedure for inspecting the condition of a tank, with special attention to sediment loads. Use the SOP log sheet to log data.

- General inspection of tank condition. Make notes as needed.
- Measurement of sediment in tank. Note deepest sediment load in millimeters. Estimate average sediment load in millimeters. Estimate percentage of tank bottom covered with sediment.

SOP 9, Step 4 Rinsing Control Tank

This step provides the procedure for rinsing a tank and removing accumulated sediment WITHOUT disinfection.

Preparation.

- PIC Reviews Tank Cleaning Procedures with contractor lead.
- Contractor supplied hoses and pressure washers are connected to fresh water supply.
- Portable pumps are staged in tank.

Rinse.

- Muck any collected silt from tank using buckets as needed.
- Thoroughly rinse all tank surfaces with generous amounts of fresh water.
- Tank is continuously pumped empty ensuring no standing water in tank bottom or piping.

Inspection.

- Tank is inspected with contractor to confirm all silt and debris is removed from tank frames and surfaces. Tank is free of collected water.
- **Any collected** solids are disposed accordance with all state and federal regulations.

SOP 9, Step 5 Rinsing and Disinfecting Treatment Tank

This step provides the procedure for rinsing a tank and removing accumulated sediment WITH disinfection.

Preparation.

- PIC Reviews Tank Cleaning Procedures with contractor lead.
- Contractor supplied hoses and pressure washers are connected to fresh water supply.
- Portable pumps are staged in tank.
- Train and outfit all tank cleaning personnel with PPE appropriate to working with 200ppm chlorine solution in confined space.
- Outfit personnel with ability to meter chlorine solution at 200ppm (approximately 1 TBSP of 5% laundry bleach solution per gallon of fresh water) into pressure washers.

Rinse and disinfect.

- Muck any collected silt from tank using buckets as needed.
- Spray chlorine solution to contact ALL horizontal and vertical surfaces of tank. Ladders shall be utilized to reach high and inaccessible places. Particular attention shall be paid to hull framing and tank top stiffeners and any other “hard to reach” locations.
- After a short contact time (not less than 5 minutes, but no more than the time it takes to spray down tank), thoroughly rinse all tank surfaces with generous amounts of fresh water.
- Thoroughly rinse all tank surfaces with generous amounts of fresh water.
- Tank is continuously pumped empty ensuring no standing water in tank bottom or piping.

Inspection.

- Tank is inspected with contractor to confirm all silt and debris is removed from tank frames and surfaces. Tank is free of collected water.
- Using “chlorine test strips” to determine residual chlorine at least six (6) random spot checks of wet surfaces do not exceed 3ppm.
- **Any collected** solids are disposed accordance with all state and federal regulations.

SOP 10 Preparation of Source Tanks (LAND-BASED)

Application	LAND-BASED	XX	SHIPBOARD	
-------------	------------	----	-----------	--

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and Online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

Source Tank Filling, Mixing, and Augmentation Overview

These combined steps describe the necessary procedure to prepare and augment Source Tanks 3-174-1 and 3-174-2 to meet required biological and water quality characteristics. These steps include tank mixing procedures to ensure that all existing and added materials remain suspended such that challenge conditions remain similar through-out the Test Cycle.

Source Tank Filling

The Source Tanks are initially filled to 98.5% full by taking a suction from the sea with the treatment pump to fill Tank 3-174-1 and Tank 3-174-2. The BWTS is bypassed during this operation.

If requirements for the organisms $\geq 50 \mu\text{m}/\text{m}^3$ size class ARE NOT met with the non-augmented seawater, then the Source Tanks are partially emptied to make room to be “topped up” with seawater that has a concentrated number of organisms in that size class.

If requirements in the $\geq 50 \mu\text{m}/\text{m}^3$ size class ARE met with the non-augmented seawater, then the tank level is left as-is, and other water quality characteristics are evaluated and augmented as needed. This includes: the 10 – 50 $\mu\text{m}/\text{m}^3$ size class, POC, DOC, and TSS concentrations.

Source Tank Mixing Overview

An air-lift mixing system, driven by the ship’s compressed air system, is used to maintain a homogeneous mixture in each Source Tank, and to keep various augmenting materials suspended throughout the augmentation process. This air-lift system is run continuously from the time of Source Tank Uptake until both Source Tanks have been discharged during the cycle uptakes.

If source tank biology or water quality is found to vary between tanks by more than 20%, either before or after augmenting, use ballast treatment pump to recirculate tanks at a slow rate until desired homogeneity is achieved. As a target, a 10% threshold of variability is desired but in all cases parameter levels must exceed challenge requirements. Alternatively, when any challenge biology or water quality condition between tanks exceeds 20% variance, the SOPs may be redlined to allow for separate (and reduced volume) control uptakes between Treatment 1 and Treatment 2 uptakes.

Source Tank Biological Augmentation Overview

If requirements for the organisms $\geq 50 \mu\text{m}/\text{m}^3$ size class are not met during filling from sea, then two organism concentrating systems are used. With the treatment piping system in sea-to-sea mode, each concentrator takes water from the main deck sampling ports, and filters the flow into a concentrate stream and an effluent stream. The concentrate streams are directed into the Source Tank accesses, while the effluent streams are directed overboard. The duration of concentrator operation is based on the starting $\geq 50 \mu\text{m}$ organism concentration.

Source Tank Water Quality Augmentation Overview

or tri-sodium citrate



DOC, POC, and TSS concentrations in the Source Tanks are augmented by adding two approved chemical components. NESTEA® “Unsweetened Decaf 100% Ice Tea Mix” is used to increase DOC concentration, while the humic material Mesa Verde Resources

Test Cycle Number

PIC Initials

Quality Initials

Date

“Micromate” is used to increase POC and TSS concentrations. The amount of each material to add is calculated from the starting concentration of DOC, POC, and TSS. The material is added to each Source Tank by first diluting in a forty-gallon container with seawater, and then pouring the mixture into the Source Tank accesses.

SOP 10, Step 1 Source Tank Filling

Operational Instructions

- Confirm Tanks 3-174-1 and 3-174-2 have been prepared as per SOP 5.
- Align pumping system to fill from the sea, past the sampling station, and by-passing the BWTS.
- Start Ballast Treatment Pump at 800 RPM 2 hours before high tide. Adjust speeds to achieve ~250m³/hr flow rate through the pump. Manage system flow rates using valve G and by varying pump speed.
- Log absolute start time, and start time relative to high tide.
- Ensure science team takes one continuous sample into Tub B3 throughout the entire uptake.
- Fill until both Source Tanks are at least 98.5% full. Secure system.
- Log absolute stop time, and stop time relative to high tide.

SOURCE TANK FILL			DATE	
TIME 24 HR (#####)	START		STOP	
	Absolute	High Tide (+/-)	Absolute	High Tide (+/-)
Tank 3-174-1				
Tank 3-174-2				

Notes: _____

SOP 10, Step 2 Source Tank Mixing

Operational Instructions

- Confirm all air fittings and connections to be securely joined and all Chicago connectors to be wired together.
- Start air compressor in S Gear Room.
- Open both manifold inlet valves, and adjust each to achieve 7 SCFM.
- Monitor flow continuously. Log flow data every 6 hours until tanks are emptied.

3-174-1	Date/ Time	Flow (SCFM)	Date/ Time	Flow (SCFM)
3-174-2	Date/ Time	Flow (SCFM)	Date/ Time	Flow (SCFM)

Notes: _____

SOP 10, Step 3 Concentration of Organisms $\geq 50 \mu\text{m}$ Size Class

The ballast piping inline sample ports are used during uptake to determine if there is an adequate concentration of live organisms in the $\geq 50 \mu\text{m}$ size class. The combined 700 cubic meter volume of the Source Tanks, including a 20% margin, requires 120,000 live organisms per cubic meter or **84,000,000 live organisms total**.

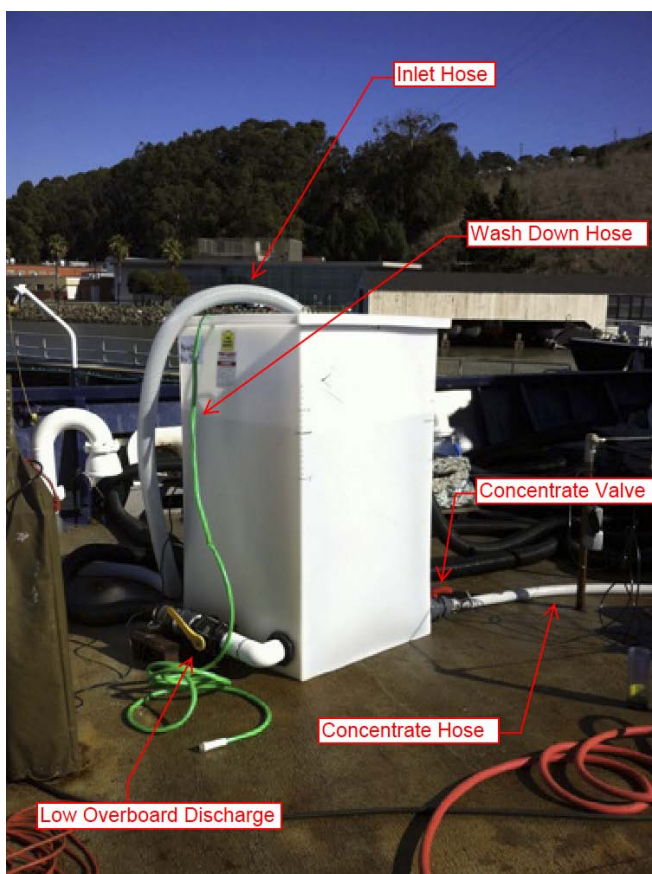
- Raw Seawater Concentration. Record the in-line concentration obtained during Source Tank Filling.
 - Initial Concentration _____ (organisms $\geq 50 \mu\text{m}/\text{m}^3$)
- Required Augmentation. Organisms will be augmented using two dedicated concentrators for each Source Tank. If the raw seawater concentration is at least 120,000 live organisms per cubic meter, then record “0” below for estimated running time. If not, then use the following Concentrator Look-up Table to fill-out below parameters.
 - Raw Water Uptake Volume (m^3): _____
 - Concentrator Make-up
 - Volume (m^3): _____
 - Estimated running time per concentrator (hours): _____
- Discharge raw water from Source Tanks until Raw Water Uptake Volume is reached.
- Ensure air lift mixing system is continuously running.
- Place concentrator online until Source Tanks are filled to 98.5%.
- Perform grab samples using Niskin bottle to determine if desired concentration (120,000 live organisms per cubic meter) has been reached.
- Repeat this entire step if the first concentration effort did not result in required organism concentration.

Concentrator Operation

The concentrators are independent of ship systems, and as such, require manual operation and supervision. The following is a general procedure for using each concentrator:

- Connect all concentrator hoses, and lower submersible pump overboard, approximately 10 ft below the waterline.
- Start the pump, and direct approximately half of the flow directly overboard using the by-pass manifold. The remaining flow should be directed into the tank.
- Once flow is established, close the low discharge and concentrate valves.
- Slowly close the by-pass valve, until all the flow is directed into the concentrator.
- When the tank is full (water level has reached the marked level on the tank wall), open the low discharge valve until steady-state water level is achieved.

- Open the concentrate valve, and establish a flow of 5 gpm (1.14 m³/hr). Quickly verify the flow rate using a 5-gallon bucket, and adjust as necessary.
- Direct the concentrate flow into the designated Source Tank.
- Periodically plug in the submersible pump and rinse down the net as necessary.
- After 4 hours, or when the net completely clogs (level differential between inside and outside of net reaches 6 inches), drain and rinse the concentrator:
 - Open the low discharge valve until the water level (inside and outside the net) begins to decrease.
 - Rinse the exposed walls of the net as the water level decreases.
 - Once the water level is below the inlet hose opening, turn off the pump. This ensures that water is not siphoned back out of the concentrator, and the net is not damaged.
 - Once the net is completely drained, remove the net and replace with a clean net. Thoroughly rinse the removed net inside and outside with freshwater.
- Run the concentrator for the estimated running time. The concentrator should be inspected frequently to ensure excessive clogging and net overflow does not occur.



Concentrator Assembly

Test Cycle Number

PIC Initials

Quality Initials

Date

Concentrator Look-up Table

Raw Water Uptake				Concentrator Make-up				Combined	
Conc.	Volume	Tank	Orgs.	Conc.	Volume	Time per Concentrator	Orgs.	Volume	Orgs.
(1000/m3)	(m3)	Filled	(1,000)	(1000/m3)	(m3)	(hours)	(1,000)	(m3)	(1,000)
120	700	98%	84000	1440	0	0	0	700	84000
115	697	98%	80155	1380	3	1	4140	700	84300
110	694	97%	76340	1320	6	3	7920	700	84300
105	690	97%	72450	1260	10	4	12600	700	85100
100	687	96%	68700	1200	13	6	15600	700	84300
95	683	96%	64885	1140	17	7	19380	700	84300
90	678	95%	61020	1080	22	10	23760	700	84800
85	673	94%	57205	1020	27	12	27540	700	84700
80	668	94%	53440	960	32	14	30720	700	84200
75	661	93%	49575	900	39	17	35100	700	84700
70	654	92%	45780	840	46	20	38640	700	84400
65	646	90%	41990	780	54	24	42120	700	84100
60	636	89%	38160	720	64	28	46080	700	84200
55	624	87%	34320	660	76	33	50160	700	84500
50	611	86%	30550	600	89	39	53400	700	84000
45	594	83%	26730	540	106	46	57240	700	84000
40	572	80%	22880	480	128	56	61440	700	84300

Assumptions

Source Tank Volume 714 cubic meters

Target Concentration 120 thousands of organisms $\geq 50 \mu\text{m}/\text{m}^3$

Minimum Live Req. 84,000 thousands of organisms required (includes 20% margin)

Concentrator 20 Live concentration ratio (concentrate to inlet)

60% Approximate average of high tide organism concentration

1.14 m³/hr concentrated effluent output rate

"Orgs." refers to number of organisms in the associated volume.

Notes: _____

SOP 10, Step 4 Augmentation of Organisms $\geq 10 \mu\text{m}$ $< 50 \mu\text{m}$

The second step of organism augmentation is the addition of phytoplankton, sizes $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$ in size. These phytoplankton are grown out from local waters in tubs located at the Facility. The tub contents are added to the tanks at the direction of the science team and the Source Tanks are again measured to assure the proper concentration has been reached.

It is important to complete this step following the concentration of organisms $\geq 50 \mu\text{m}$, as the concentration process will accumulate phytoplankton as well as zooplankton.

Water Quality Augmentation Background Information

The following steps describe the necessary procedure to augment the source tanks with various materials to meet IMO water quality standards. The Source Tanks are individually sampled via a Niskin bottle, and analyzed for salinity, Dissolved Organic Carbon (DOC) content, Particulate Organic Carbon content (POC), and Total Suspended Solids (TSS). Based on these results, amounts of supplemental materials are calculated, and then added to the Source Tanks.

Each of the following steps shall be completed for each Source Tank, 3-174-1 and 3-174-2.

SOP 10, Step 5 Water Quality Sampling

- Source Tank water is sampled continuously during filling in Step 1.
- Perform analysis for Salinity, DOC, POC, and TSS. Record below.
- Calculate (see calculation methods following) and record amount of augmentation materials to be added.

Tank	Water Quality	Criteria		Measured	To Add
		Brackish	Salt		
3-174-1	DOC (mg/L)	≥ 6	≥ 6		_____ lbs ice tea
	POC (mg/L)	≥ 5	≥ 4		_____ lbs micromate
	TSS (POC + MM) (mg/L)	≥ 50	≥ 24		_____ lbs test dust
	Salinity (PSU)	10-20	>32		_____ gal brine
3-174-2	DOC (mg/L)	≥ 6	≥ 6		_____ lbs ice tea
	POC (mg/L)	≥ 5	≥ 4		_____ lbs micromate
	TSS (POC + MM) (mg/L)	≥ 50	≥ 24		_____ lbs test dust
	Salinity (PSU)	10-20	>32		_____ gal brine

Notes: _____

SOP 10, Step 6 Water Quality Calculation

These equations include a 20% margin for concentration criteria.

Dissolved Organic Carbon (DOC)

DOC concentration is augmented with *Camellia sinensis*, which is found in NESTEA® “Unsweetened Decaf 100% Ice Tea Mix.” The amount of tea to add to each tank is determined by inserting the measured concentration of DOC (mg/L) into the following equation:

$$14.57 - [2.03 \times \text{_____} (\text{mg/L DOC})] = \text{_____} \text{ lbs ice tea (Tank 3-174-1)}$$

$$14.57 - [2.03 \times \text{_____} (\text{mg/L DOC})] = \text{_____} \text{ lbs ice tea (Tank 3-174-2)}$$

Particulate Organic Carbon (POC) and Total Suspended Solids (TSS)

Both POC and TSS are augmented with humic material, which is found in Mesa Verde Resources “Micromate.” TSS is made up in part by POC, so Micromate will be used to augment the level of POC, and simultaneously increase the level of TSS. Ice tea also contributes to POC concentration, so the weight of ice tea calculated above also impacts the required weight of Micromate to be added. The amount of Micromate to add to each tank is determined by inserting the measured concentration of POC (mg/L), and the weight of ice tea calculated above, into the following equation:

$$9.03 - [1.50 \times \text{_____} (\text{mg/L POC})] - [0.19 \times \text{_____} (\text{lbs ice tea})] = \text{_____} \text{ lbs Micromate (Tank 3-174-1)}$$

$$9.03 - [1.50 \times \text{_____} (\text{mg/L POC})] - [0.19 \times \text{_____} (\text{lbs ice tea})] = \text{_____} \text{ lbs Micromate (Tank 3-174-2)}$$

Arizona Test Dust to Supplement TSS

If additional TSS is measured to be below the required level after the addition of Micromate, Arizona Test Dust can be used to meet criteria. The amount of Test Dust to add to each tank is determined by inserting the post-Micromate measured concentration of TSS into the following equation:

$$98.57 - [1.6424 \times \text{_____} (\text{mg/L TSS})] = \text{_____} \text{ lbs Test Dust (Tank 3-174-1)}$$

$$98.57 - [1.6424 \times \text{_____} (\text{mg/L TSS})] = \text{_____} \text{ lbs Test Dust (Tank 3-174-2)}$$

Notes: _____

SOP 10, Step 7 Augmentation to Tanks

*****water quality augmentation may occur simultaneously with running concentrator for biological augmentation*****

After finding the weight of ice tea and Micromate to augment the source tanks with, the materials are diluted in saltwater from the treatment piping system, and then added to the mixing source tanks. Protective dusk mask, eye protection, and gloves should be used when handling Micromate.

Ice Tea Tank Delivery

- Measure the above calculated weight of ice tea mix for Tank 3-174-1, and distribute into the bottom of a clean, open-topped ~forty gallon container in several increments. Fill container with seawater with the concentrator rinse hose and pump until full. Ten (10) bottles equals approximately 2 lbs of ice tea.
- Empty container contents into Tank 3-174-1.
- Repeat ice tea diluting and delivery with Tank 3-174-2.

Micromate Tank Delivery

- Measure the above calculated weight of Micromate into for Tank 3-174-1 and distribute into the bottom of a clean, open-topped ~forty gallon container in several increments. Fill container with seawater with the concentrator rinse hose and pump until full.
- Empty container contents into Tank 3-174-1.
- Repeat Micromate diluting and delivery with Tank 3-174-2.

Arizona Test Dust Tank Delivery

- Measure the above calculated weight of Test Dust into for Tank 3-174-1 and distribute into the bottom of a clean, open-topped ~forty gallon container in several increments. Fill container with seawater with the concentrator rinse hose and pump until full.
- Empty container contents into Tank 3-174-1.
- Repeat Test Dust diluting and delivery with Tank 3-174-2.

Notes: _____

SOP 10, Step 8 Final Biology and Water Quality Sample

- Take three samples from the top, middle, and bottom of the two Source Tanks' upper area through main deck access hatches (total three samples each). Different depths are sampled to confirm tank homogeneity as well as criteria requirements.
- For each sample, use a peristaltic pump to retrieve an adequate sample volume from the required depth, or lower a Niskin bottle to required depth, close Niskin bottle, and retrieve from tank.
- Evaluate water sample for organisms $\geq 50 \mu\text{m}$, organisms $\geq 10 < 50 \mu\text{m}$, POC, DOC, and TSS. **TSS, DOC, & POC are estimated due to assay time and augmented accordingly.**
- If additional organisms or materials are required, the tanks must be sampled again AFTER these adjustments have been made.
- Log the average biological and water quality results for each tank in the measured column of the table below.
- If the two tank's contents differ significantly, they will be homogenized via a designated pumping operation that will move water between the two tanks.

Source hold time is minimized and treatment pump recirculation is used to homogenize

Tank	Source Living Populations	Criteria		Measured		
		Brackish	Salt	Top	Middle	Bottom
3-174-1	≥ 50 microns (organisms/m ³)	10 ⁵	10 ⁵			
	$\geq 10 < 50$ microns (organisms/mL)	10 ³	10 ³			
	< 10 microns (bacteria/mL)	10 ⁴	10 ⁴			
	Water Quality	Criteria	Criteria	Measured	Measured	Measured
		Brackish	Salt	Top	Middle	Bottom
	Salinity (PSU)	10-20	>32			
	DOC (mg/L)	≥ 6	≥ 6			
	POC (mg/L)	≥ 5	≥ 4			
	TSS (mg/L)	≥ 50	≥ 25			
Tank	Source Living Populations	Criteria		Measured		
		Brackish	Salt	Top	Middle	Bottom
3-174-2	≥ 50 microns (organisms/m ³)	10 ⁵	10 ⁵			
	$\geq 10 < 50$ microns (organisms/mL)	10 ³	10 ³			
	< 10 microns (bacteria/mL)	10 ⁴	10 ⁴			
	Water Quality	Criteria	Criteria	Measured	Measured	Measured
		Brackish	Salt	Top	Middle	Bottom
	Salinity (PSU)	10-20	>32			
	DOC (mg/L)	≥ 6	≥ 6			
	POC (mg/L)	≥ 5	≥ 4			
	TSS (mg/L)	≥ 50	≥ 25			

SOP 11 Calibration of Sampling System Flow Meter

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

All flow meters have been factory calibrated with a “K” constant pertaining to a specific flow meter. Each flow meter will be field calibrated once per year by passing a known volume of seawater past the sensor. This volume will be compared to the factory calibration volume and should match the calibration within 10%. If it does not, we will send the sensor back for factory calibration.

Weekly calibration monitoring will take place by validating measured volumes against graduated marks on sample tubs.

SOP 12 Calibration of Sea-Bird Thermosalinograph (TSG)

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

The Sea-Bird Thermosalinograph (TSG) is a factory calibrated unit received on site in November 2010. It will be packaged and sent back to the factory for calibration on a routine basis. The unit will be monitored in operation for consistent data and calibrated on the following schedule:

- Every six months, send unit to factory for full calibration and certification of all parameters.
- Prior to each test, run system and verify that water quality data is within expected range for challenge water.
- Following each test, compare collected data with results from grab sample analysis.

Maintain a calibration log of TSG status.

SOP 13 Calibration of Flow Cytometer & Lab Fluororometer

Application

LAND-BASED

XX

SHIPBOARD

XX

SOP 13, Step 1 Accuri C6 Flow Cytometer Calibration

Validating the Performance of the C6

Perform a validation of the fluidics at least once each day that you use the C6. This ensures that the C6 is working properly before running experimental samples. Use the same CFlow file each day to collect validation bead data so that you can compare trends over time. When you run a validation, advance to the next empty well in row A-D (for 8-peak beads) or E-H (for 6-peak beads). Include the date in the *Sample Naming Field* when you name the sample to keep track of each day's validation. Start a new validation bead file when you fill up the wells. Reagents required:

- Spherotech 8-Peak Validation Beads (Accuri Part# QA-100, supplied with the initial C6 shipment)
- Spherotech 6-Peak Validation Beads (Accuri Part# QA-110, supplied with the initial C6 shipment)
- Sheath fluid: De-ionized, filtered water (0.2 µm filter) plus Bacteriostatic Concentration Solution (Accuri Part# KR-220, supplied with the initial C6 shipment)

Running Validation Beads

To run the validation beads:

Setup:

- If this is the first time using the C6 Cytometer, open a CFlow file and run filtered de-ionized water from a 12x75 mm sample tube for at least 15 minutes.
- Verify that the file named CFlow 8 & 6 Peak Bead Template.c6t has been copied to the CFlow computer. The file is located on the CFlow CD or flash drive and is on the Accuri website
- (www.accuricytometers.com/technical_information/templates/).
- Select File > Open CFlow File or Template.
- In the Open dialog box, browse to the location of the template file and open the file.

Clean the SIP:

- Click on the first empty well in rows A-D to advance to the well.
- Place an empty 12x75 mm tube on the SIP.
- Click on the Backflush button.
- After the backflush, place a fresh tube with 2 mL of filtered, de-ionized water on the SIP.
- Disable the Run Unlimited check box in the Instrument Control Panel.
- Enable the Time check box next to the Min and Sec fields in the Instrument Control Panel and type in a run time of two minutes.
- Select the Fast radio button in the Fluidics section of the Control Panel.

- Click on the RUN button to rinse out the SIP.
- Once the run is finished, click on the Delete Sample Data button to delete data collected during the rinse.
- Remove the tube from the SIP.

Run 8-Peak Validation Beads:

- Disable the Time check box next to the Min and Sec fields and enable the Events check box to stop the run when the specified number of events has been acquired.
- Type 50000 in the events edit box and select Ungated Sample from the associated drop-down list.
- Vortex a sample tube containing suspended 8-peak validation beads, prepared according to the package instructions. Place the tube on the SIP.
- Select the Slow radio button in the Fluidics section of the Control Panel.
- Click on the RUN button to start acquisition. Acquisition automatically stops after 50,000 total events are acquired.

CAUTION: Make sure the well in CFlow is empty before starting the run. If the button displays ADD TO, the well already contains data.

NOTE: The R1 region may not encompass the main population of bead events on the FSC-H vs. SSC-H plot. This is common and acceptable at this stage.

- Name the sample by typing a name in the text box just above the Sample Grid.
- Include the date in the sample name to differentiate it from samples collected on other dates.

NOTE: You can also name samples before, during, or after collection.

- When the collection is finished, remove the sample tube and wipe off the end of the SIP with a lint-free tissue (or similar material) to minimize sample carryover.

Run 6-Peak Validation Beads:

- Vortex a tube of suspended 6-peak validation beads, prepared according to the package instructions. Place the tube on the SIP.
- Click on the first empty well in rows E-H to advance to the well.
- Verify that Events is still enabled and set at 50,000 in Ungated Sample.
- Click on the RUN button.

NOTE: The R2 region may not encompass the main population of bead events on the FSC-H vs. SSC-H plot. This is common and acceptable at this stage.

- Name the sample with a name that includes the date processed.

End The Procedure:

- When the collection is finished, remove the sample tube from the SIP and wipe off the end of the SIP with a lint-free tissue.
- Place a tube with 2 mL of filtered, de-ionized water on the SIP and advance to any empty data well.

- Select the Time check box (Min Sec) in the Instrument Control Panel and set it
- for two minutes.
- Click on the *RUN* button.
- When the run is finished, leave the tube on the SIP.

SOP 13, Step 2 FluoroMax-2 Protocol

Startup

- Turn on lamp
- Turn on power
- Turn on computer

Software

- Double click on Instrument Control Center (ICC) icon on desktop
- Instrument will initialize and then prompt :bring hardware to last position?" – click on YES

Perform a lamp scan:

- Click on Applications/Experiment/Collect/Experiment
- Click on Experiment in the upper left hand corner, open c:\datamax\LAMP.EXP
- Make sure there is no sample cuvette in the machine
- Name the data file
- Click RUN
- The screen will display sloppy spectrum. The highest peak should be at 467 +_ 0.5nm (intensity around 0.01 – 0.05 cps). If outside the range, the excitation monochrometer must be calibrated. Be sure to record the observed excitation λ , as you will need it for the next step.

To calibrate excitation monochrometer:

- Exit current window
- Go to ICC window and click on Applications\Real Time Display
- Under Monos Increment (nm), fill in the observed λ from your lamp scan in the box next to EX1
- Click the small EX1 box to set the value. Close this menu
- Go to the ICC window and click on the Run Visual Set Up icon
- Click on the left yellow bar, which is the instrument's excitation monochrometer grating control
- Click calibrate, enter the desired lamp scan value (467), then click 'OK'

- Close grating window and visual setup window
- Run another lamp scan to confirm calibration

Perform a raman water scan:

- Go to Collect and click Experiment
- Open the experiment folder, c:\datamax\raman.exp (or ramnw.exp)
- Place the quartz, rectangular, 4-sided cuvette containing milli-Q into the instrument
- Click RUN
- The screen will display a spectrum with one large peak. The peak should be $\lambda 397 \pm 0.5\text{nm}$ (intensity approximately 150,000 cps). If outside this range, the emission monochromator must be calibrated. Once again, λ .

To Calibrate Emission Monochromator

- Follow same procedure for the excitation monochromator except, on the Real Time Display page, record observed em λ of your raman scan in the box next to EM1
- Similarly, click on the Run Visual Set Up icon, click the right yellow bar, emission monochromator grating control
- Click 'calibrate', enter desired raman scale value (397), then click 'OK'
- Run another raman scan to confirm calibration

Shut Down

- Save all data
- Shut down computer
- Turn off lamp
- Turn off power

SOP 14 Operation of BWTS and Facility Piping System (LAND-BASED)

Application	LAND-BASED	XX	SHIPBOARD	
-------------	------------	----	-----------	--

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Test Cycle Piping Diagrams identify system process points, instrumentation locations, and designate valve names. They are available in the automation system, on Facility laminated placards, and in key documents.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

Uptake Background Information

and then the control tank(s)
5-104-1&2

Ballast water uptake includes one event that uptakes to Treatment Tank 1 (3-154-1) ~~and then the Control Tank (6-E-0)~~, and a second event that uptakes to Treatment Tank 2 (3-154-2). This sequence allows the one control tank to serve both treatment tanks. ~~or recirculation~~

The first event primes the BWTS inlet piping, starts the BWTS, and passes ballast water from the seachest through the BWTS and then overboard (sea-to-sea). The sea-to-sea mode enables the sampling team to set-up the sampling system. The pumping team then: switches suction to the Source Tanks, fills Treatment Tank 1 with treated water. ~~When Treatment Tank 1 is full, the team switches to start fill of the Control Tank while shutting down the BWTS, and continues until Control Tank is full. Pumping is then stopped to clean the piping system of control ballast water. After the brief stop for cleaning,~~ Treatment Tank 2 is filled in the same manner as Treatment Tank 1. **Control is filled after treatment 2.**

Communications between the pumping team (opening and closing valves, operating the treatment system pump, and operating the BWTS) and the sampling team is performed by radio, visual communications, and the automation system ballast order telegraph (BOT). Of these, the BOT is the primary and definitive means of communication.

The sampling team will prepare the sampling system by flushing all equipment, hoses, and other devices with raw seawater before uptake. During set-up, the sampling system will also balance flows into the tubs. However, the sampling team will only fill the tubs when directed by the pumping team. Uptake samples are collected in unfiltered volumes and homogenized for analysis.

Please reference the Land-Based Piping Diagrams in SOP 2.

SOP 14, Step 1 Pipe Flushing

Prior to uptakes events, the Facility piping system is flushed with bleach solution. Flush pump loop, 01 deck loop, Source Tank, Tank 3-154-1, Tank 3-154-2, and Tank 6-E-0 branch lines. Drain Facility piping.

Pipe Flushing Set-Up

- Secure tank bleeds at Valves V, T, and W.
- Connect and open “City Water” to pump suction.
- Connect bleach tank to pump suction. Add one (1) gallon of 5% bleach solution and mix with fresh water.
- Open pump loop Valves C, K, and M. Throttle L.
- Open D, E, F1, F2, and 1-1/2 inch vent valve at 01 Deck and vent air to fill system. Note: May require periodic venting during flush cycles.
- Throttle valve L to create a vacuum at pump suction and draw bleach solution into piping system.

Pump Loop Flushing

- Start pump at 800 RPM. Using valve L as per above, draw and flush bleach solution into system. Vary throttle valve L and pump speed to maintain ~200 m³/hr of flow through the pump. Run minimum of five minutes.
- Log valve positions and log “Pump Loop Flushed.”

01 Deck Loop and Tank Branch Line Flushing

- Open valves P and J.
- Open throttle valve G to 2 threads. Close valve L, while adjusting valve G and pump speed to maintain 150-200 m³/hr flow rate in Tank 3-154-1 branch. Run minimum of five minutes.
- Log valve positions and “01 Deck Loop Flushed” and “3-154-1 Branch Flushed.”
- Open valves H and N. Close valves P and J. Run minimum of five minutes.
- Log valve positions and “3-154-2 Branch Flushed.”
- Open valves ZR, ZP, ZJ, A, and B. Close valves M, N, and H. Run minimum of five minutes.
- Log valve positions and “Control Branch Flushed.”

Secure System

- Secure pump, city water, and close valves ZR, ZP, ZJ, and A. Open the following bleeds: D, E, H-N-V, J-P-T, and ZP-ZR.
- Drain all Facility piping through low-point drains using vents and system valves.
- Secure all valves. Log “Facility Piping Drained.”

Test Cycle Number

PIC Initials

Quality Initials

Date

Pump Loop Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks				Treat. Tanks		Control		Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

01 Deck and 3-154-1 Branch Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks				Treat. Tanks		Control		Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

01 Deck and 3-154-2 Branch Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V		ZW	Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

01 Deck and Control Branch Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V		ZW	Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

SOP 14, Step 2 Uptake Sea-to-Sea Mode or recirculation

- Confirm Tanks 3-154-1, 3-154-2, and 6-E-0 are empty and clean.
- Confirm Source Tanks 3-174-1 and 3-174-2 are full, meet challenge water conditions, and air lift mixers are operating.
- Align valves and bleeds as per diagram. Keep valves E and R closed, and throttle valve G to three (3) threads showing. or use recirculation line-up
- Confirm 01 Deck by-pass is double blocked and bled.
- Open valves L, K, M, and ZY to provide pump recirculation flow until BWTS is running.
- Start Ballast Treatment Pump at low RPM. Throttle valve L and bleed air from system at BWTS inlet vent.
- Turn on BWTS, set to Ballast Mode.
- Once BWTS is warmed up and online it will automatically open its outlet valve. Slowly open valve E and bleed air. Once line is charged, slowly open valve R, allowing treated ballast water to flow through discharge piping to the overboard. or recirculation
- Close valves L, K, and M.
- Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Place Water Quality Monitors online.
- Alert sampling team "Uptake Sea to Sea" via Ballast Order Telegraph (BOT). The sampling team will prepare for sampling. Once the system is flushed, the sample team will return "Ready to Sample" via BOT. Sampling team tasks:
 - Flush sampling system with raw seawater (from inlet to BWTS system).
 - Flow sample water into sample tubs.
 - Start water quality probe(s) in sample tub(s).
 - Prepare 170L container at BWTS outlet.
- Log piping system status (ballast treatment pump, BWTS, valves, and bleeds).

Test Cycle Number

PIC Initials

Quality Initials

Date

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")			(Open Valves Mark "O")			(Pull Plug, Open Valve Mark "OUT")			
Pump			Device (BWTS)			Bleed Plug & Valve		Location Status	
A	B	C	D	E	F1	F2	G	Seachest A-B	
Pump Manifold			Utility Manifold			Source Tanks		W-ZT	
N	P	R	H	J	K	L	M	Treatment 1 Tank J-P-T	
Source Tanks			Treat. Tanks		Control	Treatment 2 Tank		H-N-V	
ZV	ZX	W	ZT	T	V	ZW	Device By-pass	F1-F2	
Skin Valves		Drain Valves		Fire/Ballast Pump		Suct	Fire/Ballast Pump	ZF-ZG	
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	
Ballast Main Piping						Ovbd	Ballast Main - Tank	ZK-ZL	
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	
								ZM-ZN	

SYSTEM PARAMETERS			TIME Use 24 HR (####)			Tank 3-154-1
Position	#1	#2	#4	#5	#6	
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 14, Step 3 Treatment 1 Uptake

- Ensure Facility is ready for uptake:
 - Facility piping system and BWTS is operating within valid test requirements.
 - Sampling team is “Ready to Sample” via BOT.
- Send discharge to Treatment Tank 1. Open valves P and T to Tank 3-154-1, then close valve R to overboard. **or isolate recirculation**
- Take suction from Source Tank 1. Open valves ZV, W, and ZT to Tank 3-174-1, then close valve B to seachest. Valve B may be left open or throttled as needed to augment ballast water volume or flow rates.
- Alert sampling team “Treatment 1 Uptake” via BOT. Sampling team tasks:
 - Flow sample water into designated tubs.
 - Monitor water quality probe(s) in sample tub(s).
 - Flow sample water into the 170L container at BWTS outlet.
- Throttle valve G and use pump’s flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data.

Once Treatment Tank 1 (3-154-1) has been filled to a volume not-less-than 200m3, the BWTS is secured, and the pumping system is secured.

- Confirm Treatment Tank 1 (3-154-1) volume is not-less-than 200 m3.
- Secure BWTS and Facility pump
 - Select BWTS Stop through automation system interface.
 - Stop treatment pump and immediately close valves P and T. This ensures that the BWTS remains flooded during its shutdown cycle.
 - Secure all remaining valves left open.
- Confirm that the BWTS has completed its shut-down cycle.

								(Installed Plug, Shut Valve Mark "IN")		
(Shut Valves Mark "X")			(Open Valves Mark "O")					(Pull Plug, Open Valve Mark "OUT")		
Pump			Device (BWTS)					Bleed Plug & Valve	Location	Status
A	B	C	D	E	F1	F2	G			
Pump Manifold			Utility Manifold					Seachest	A-B	
N	P	R	H	J	K	L	M	Source Tanks	W-ZT	
Source Tanks			Treat. Tanks		Control	<div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></</div></div>				

Test Cycle Number

PIC Initials

Quality Initials


Date

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-1
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 14, Step 4 Uptake Sea-to-Sea Mode or recirculation

- Align valves and bleeds as per diagram. Keep valves E and R closed, and throttle valve G to three (3) threads showing. or use recirculation line-up
- Confirm 01 Deck by-pass is double blocked and bled.
- Open valves L, K, and M to provide pump recirculation flow until BWTS is running.
- Start Ballast Treatment Pump at low RPM. Throttle valve L and bleed air from system at BWTS inlet vent.
- Turn on BWTS, set to Ballast Mode.
- Once BWTS is warmed up and online it will automatically open its outlet valve. Slowly open valve E and bleed air. Once line is charged, slowly open valve R, allowing treated ballast water to flow through discharge piping to the overboard. or recirculation
- Close valves L, K, and M.
- Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Place Water Quality Monitors online.
- Alert sampling team "Uptake Sea to Sea" via Ballast Order Telegraph (BOT). The sampling team will prepare for sampling. Once the system is flushed, the sample team will return "Ready to Sample" via BOT. Sampling team tasks:
 - Flush sampling system with raw seawater (from inlet to BWTS system).
 - Flow sample water into sample tubs.
 - Start water quality probe(s) in sample tub(s).
 - Prepare 170L container at BWTS outlet.
- Log piping system status (ballast treatment pump, BWTS, valves, and bleeds).

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN")	
								(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZO	ZR	U	Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials


Date

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-2
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 14, Step 5 Treatment 2 Uptake

- Ensure Facility is ready for uptake:
 - Facility piping system and BWTS is operating within valid test requirements.
 - Sampling team is “Ready to Sample” via BOT.
- Send discharge to Treatment Tank 2. Open valves N and V to Tank 3-154-2, then close valve R to overboard. **or isolate recirculation**
- Take suction from Source Tank 2. Open valves ZX, W, and ZT to Tank 3-174-2, then close valve B to seachest. Valve B may be left open or throttled as needed to augment ballast water volume or flow rates.
- Alert sampling team “Treatment 2 Uptake” via BOT. Sampling team tasks:
 - Flow sample water into designated tubs.
 - Monitor water quality probe(s) in sample tub(s).
 - Flow sample water into the 170L container at BWTS outlet.
- Throttle valve G and use pump’s flow control mode to maintain 250 m3/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data.
- (See next step for switching system once tank has reached at least 200 m3 volume.)

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN")	
								(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials


Date

SYSTEM PARAMETERS			TIME Use 24 HR (####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-2
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 14, Step 6 Control Uptake

Once the Treatment Tank 2 (3-154-2) has been filled to a volume not-less-than 200 m³, the system is switched to fill the Control Tank (~~6-E-0~~) and the BWTS is secured.

- Ensure Facility is ready for Control Uptake: 
 - Treatment Tank 2 (3-154-2) volume is not-less-than 200 m³.
 - Facility piping system and BWTS is operating within valid test requirements.
 - Sampling team is “Sampling” via BOT.
 - Alert sampling team via radio to prepare for Control Uptake.
- Confirm sampling team is ready via radio.
- Send discharge to Control Tank.
 - Open valves ZR, ZP and ZW, then close valves N and V to begin filling Tank 6-E-0 and stop filling Tank 3-154-2.
 - Immediately open plugged drain between valves N and V at tank pipe inlet – double block and bleed.
- Secure BWTS.
 - Open 01 Deck by-pass valves F1 and F2.
 - Throttle valve G and use pump’s flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
 - Select BWTS Stop through automation system interface.
 - Once the BWTS system is completely shutdown, isolate by closing valves D and E.
 - Throttle valve G and use pump’s flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Alert sampling team “Control Uptake” via BOT. Sampling team tasks:
 - Switch flow of sample water to designated sample tubs.
 - Switch water quality probe(s) to designated sample tub(s).
 - Stop sample water to designated 170L container at BWTS outlet.
- Log Valve and System Data.

Once Source Tank 2 (3-174-2) has reached a level of ~300 mm (1 ft), Suction is then switched to Source Tank 1 (3-174-1).

- When Source Tank 2 (3-174-2) innage is at 300 mm (1 ft), open Source Tank 1 (3-174-1) root valve ZV, and then close Source Tank 2 (3-174-2) root valve ZX.
- Throttle valve G and use pump’s flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data.

Once Control Tank (~~6-E-0~~) has been filled to a volume not-less-than 200 m³, the pumping system is secured.

- Stop treatment pump.
- Flow automatically stops at the sampling system.
- Secure all remaining valves left open.

Log during Control Uptake from Source Tank 2

Test Cycle Number

PIC Initials

Quality Initials

Date

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")			(Open Valves Mark "O")					(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

SYSTEM PARAMETERS			TIME Use 24 HR (####)			
Position	#1	#2	#4	#5	#6	Tank 6-E-0
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Log during Control Uptake from Source Tank 1

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

SYSTEM PARAMETERS			TIME Use 24 HR (####)			
Position	#1	#2	#4	#5	#6	Tank 6-E-0
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 14, Step 7 Tank Holding and Pipe Flushing

Treatment 1, Treatment 2, and Control tanks are held for not-less-than 120 hours.

After filling and before discharging, the Facility piping system is flushed with bleach solution. Flush pump loop, 01 deck loop, Source Tank, Tank 3-154-1, Tank 3-154-2, and Tank 6-E-0 branch lines. Drain Facility piping.

Tank Holding and Monitoring

Once the uptake events are completed, perform the following.

- Ensure Facility piping system is secured.
 - Sound tanks to confirm volumes.
 - Ensure security of ballast treatment pump, BWTS, and all valves.
 - Ensure all valves isolating Treatment Tank 1 (1-54-1) and Treatment Tank 2 (1-154-2) are isolated by double block and bleed.
 - Log condition of piping system (pump, BWTS, valves and bleeds).
- Monitor tanks during holding period.
 - Treatment Tank 1 (1-154-1), Treatment Tank 2 (3-154-2), and Control Tank (6-E-0) for not-less-than 120 hours (Start to Start). This means if Treatment Tank 1 Uptake started on Monday at 0800 hours, then Treatment Tank 1 discharge is to start no sooner than Saturday at 0800 hours.
 - Log tank data every 12 hours.

								(Installed Plug, Shut Valve Mark "IN")		
(Shut Valves Mark "X")			(Open Valves Mark "O")					(Pull Plug, Open Valve Mark "OUT")		
Pump			Device (BWTS)					Bleed Plug & Valve	Location	Status
A	B	C	D	E	F1	F2	G	Seachest	A-B	
Pump Manifold			Utility Manifold					Source Tanks	W-ZT	
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T	
Source Tanks			Treat. Tanks		Control	<div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div></div>		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V	ZW	<div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div></div>	Device By-pass	F1-F2	
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG	
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR	
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL	
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN	

Test Cycle Number

PIC Initials

Quality Initials

Date

Tank 3-154-1	Date/ Time	Temp (C)	Volume (m3)	Date/ Time	Temp (C)	Volume (m3)

Tank 3-154-2	Date/ Time	Temp (C)	Volume (m3)	Date/ Time	Temp (C)	Volume (m3)

5-104-1&2

Tank 6-E-0	Date/ Time	Temp (C)	Volume (m3)	Date/ Time	Temp (C)	Volume (m3)

Notes: _____

Pipe Flushing Set-Up

- Secure tank bleeds at Valves V, T, and W.
- Connect and open “City Water” to pump suction.
- Connect bleach tank to pump suction. Add one (1) gallon of 5% bleach solution and mix with fresh water.
- Open pump loop Valves C, K, and M. Throttle L.
- Open D, E, F1, F2, and 1-1/2 inch vent valve at 01 Deck and vent air to fill system. Note: May require periodic venting during flush cycles.
- Throttle valve L to create a vacuum at pump suction and draw bleach solution into piping system.

Pump Loop Flushing

- Start pump at 800 RPM. Using valve L as per above, draw and flush bleach solution into system. Vary throttle valve L and pump speed to maintain ~200 m³/hr of flow through the pump. Run minimum of five minutes.
- Log valve positions and log “Pump Loop Flushed.”

01 Deck Loop and Tank Branch Line Flushing

- Open valves P and J.
- Open throttle valve G to 2 threads. Close valve L, while adjusting valve G and pump speed to maintain 150-200 m³/hr flow rate in Tank 3-154-1 branch. Run minimum of five minutes.
- Log valve positions and “01 Deck Loop Flushed” and “3-154-1 Branch Flushed.”
- Open valves H and N. Close valves P and J. Run minimum of five minutes.
- Log valve positions and “3-154-2 Branch Flushed.”
- Open valves ZR, ZP, ZJ, A, and B. Close valves M, N, and H. Run minimum of five minutes.
- Log valve positions and “Control Branch Flushed.”

Secure System

- Secure pump, city water, and close valves ZR, ZP, ZJ, and A. Open the following bleeds: D, E, H-N-V, J-P-T, and ZP-ZR.
- Drain all Facility piping through low-point drains using vents and system valves.
- Secure all valves. Log “Facility Piping Drained.”

Test Cycle Number

PIC Initials

Quality Initials

Date

Pump Loop Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V		ZW	Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

01 Deck and 3-154-1 Branch Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V		ZW	Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

01 Deck and 3-154-2 Branch Flushing Log

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN") (Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks				Treat. Tanks		Control		Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

01 Deck and Control Branch Flushing Log

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN") (Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks				Treat. Tanks		Control		Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

Recirculation and Discharge Background Information

Ballast water discharge proceeds following a holding time of not-less-than 120 hours.

Discharge includes three events that discharge Treatment Tank 1 (3-154-1), Treatment Tank 2 (3-154-2), and Control Tank (~~6-E-0~~). **5-104-1&2**

The first event primes the BWTS inlet piping, starts the BWTS, and takes suction from Treatment Tank 1. The treated water passes the BWTS, and returns to the treatment pump suction (recirculation). The recirculation mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge to overboard, emptying Treatment Tank 1 to sea, while the sampling team takes triplicate sampling of the treated water. In the second event, Treatment Tank 2 is discharged in the same manner as Treatment Tank 1. **The third event must follow the discharge of both Treatment Tanks.** The Control Tank is recirculated, now by-passing the BWTS. The untreated control water is then sampled and discharged to sea.

Communication between the pumping team and sampling team is performed as it was for uptake procedures, where ballast order telegraph (BOT) is the primary and definitive means of communication. Sampling procedures are similar to that of uptake, but now require triplicate sampling during each discharge event.

The sampling team will prepare the sampling system by flushing all equipment, hoses, and other devices with held water before discharge. During set-up, the sampling system will also balance flows into the tubs where the plankton nets are rigged. However, the sampling team will only send ballast water through the plankton nets when directed by the pumping team. This is called “netting.” Only during an actual discharge are the samples netted, in order to maximize the sample representativeness of the ballast tank contents.

SOP 14, Step 8 Treatment Tank 1 – Discharge Recirculation

*****Both Treatment Tanks must be discharged BEFORE Control Tank*****


- Confirm Tanks 3-154-1, 3-154-2, and 6-E-0 have been held for no-less-than 120 hours.
- Align valves and bleeds as per recirculation diagram. Throttle valve G to three (3) threads showing.
- Confirm 01 Deck by-pass is double blocked and bled.
- Open valves L and K to provide pump recirculation flow until BWTS is running.
- Start Ballast Treatment Pump at low RPM. Throttle valve L and bleed air from system at BWTS inlet vent. with filter backwash directed to ballast tank.
- Turn on BWTS, ~~set to De-Ballast Mode.~~ Allow BWTS to warm up.
- Once BWTS is warmed up and online it will automatically open its outlet valve, allowing treated ballast water to recirculate to the treatment pump.
- Close valves L and K.
- Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Place Water Quality Monitors online.
- Alert sampling team "Treatment Recirculation" via BOT. The sampling team will set flow rates, and alert via radio to Discharge to flush system.
- Open valve R and close valve P, await signal from sampling team.
- Once the system is flushed, the sampling team will alert via radio to return to recirculation. Open valve P and close valve R.
- The sampling team will prepare the system. Once the sampling system is prepared, the sampling team will return "Ready to Sample" via BOT. Sampling team tasks:
 - Flow sample water into sample tubs, but not through plankton nets.
 - Fill the sample tubs to the appropriate level.
 - Start water quality probes in sample tubs.
- Log piping system status (ballast treatment pump, BWTS, valves, bleeds).
















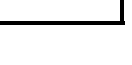
Test Cycle Number

PIC Initials

Quality Initials

Date

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")			(Open Valves Mark "O")			(Pull Plug, Open Valve Mark "OUT")			
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G		
Pump Manifold			Utility Manifold					Seachest	A-B
N	P	R	H	J	K	L	M	Source Tanks	W-ZT
Source Tanks			Treat. Tanks		Control			Treatment 1 Tank	J-P-T
ZV	ZX	W	ZT	T	V	ZW		Treatment 2 Tank	H-N-V
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Device By-pass	F1-F2
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Fire/Ballast Pump	ZF-ZG
Ballast Main Piping							Ovbd	Ballast Main - Pump	ZP-ZR
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZK-ZL
								Ballast Main - Tank	ZM-ZN

SYSTEM PARAMETERS			TIME Use 24 HR (####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-1
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 14, Step 9 Treatment Tank 1 Discharge

- Ensure Facility is ready for discharge:
 - Facility piping system and BWTS is operating within valid test requirements.
 - Sampling team is “Ready to Sample” via BOT.
- Send discharge to overboard. Open valve R and close valve P.
- Alert sampling team “Treatment 1 Discharge” via BOT. Sampling team tasks:
 - Flow sample water through designated plankton nets.
 - Monitor water quality probes in sample tubs.
- Throttle valve G and use pump’s flow control mode to maintain 250 m3/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data. **Monitor and log filter backwash pressure.**

Once Treatment Tank 1 (3-154-1) has reached a level of ~300 mm (1 ft), the flow rate is reduced until tank is empty. At loss of suction, the BWTS is secured, and the pumping system is secured.

- Slow pumping rate to 50 m3/hr when Treatment Tank 1 (3-154-1) innage is at 300 mm (1 ft).
- When ballast treatment pump loses suction, secure the BWTS and treatment pump.
 - Select BWTS Stop through automation system interface.
 - Stop treatment pump and immediately close valve R to overboard. This ensures that the BWTS remains flooded during its shutdown cycle.
 - Secure all remaining valves left open.
- Confirm that the BWTS has completed its shut-down cycle.

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN")	
Pump				Device (BWTS)				(Pull Plug, Open Valve Mark "OUT")	
A	B	C		D	E	F1	F2	G	
Pump Manifold				Utility Manifold					
N	P	R		H	J	K	L	M	
Source Tanks				Treat. Tanks		Control			
ZV	ZX	W	ZT	T	V	ZW			
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct		
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY		
Ballast Main Piping							Ovbd		
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U		
								Bleed Plug & Valve	Location Status
								Seachest	A-B
								Source Tanks	W-ZT
								Treatment 1 Tank	J-P-T
								Treatment 2 Tank	H-N-V
								Device By-pass	F1-F2
								Fire/Ballast Pump	ZF-ZG
								Ballast Main - Pump	ZP-ZR
								Ballast Main - Tank	ZK-ZL
								Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials

Date

SYSTEM PARAMETERS			TIME Use 24 HR (####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-1
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

B/W Press (cycle)

#1

#2

#3

#4

#5

#6

Notes: _____

SOP 14, Step 10 Treatment Tank 2 Discharge Recirculation

*****Both Treatment Tanks must be discharged BEFORE Control Tank*****


- Align valves and bleeds as per recirculation diagram. Throttle valve G to three (3) threads showing.
- Confirm 01 Deck by-pass is double blocked and bled.
- Open valves L and K to provide pump recirculation flow until BWTS is running.
- Start Ballast Treatment Pump at low RPM. Throttle valve L and bleed air from system at BWTS inlet vent. with filter backwash directed to ballast tank.
- Turn on BWTS, ~~set to De-Ballast Mode~~. Allow BWTS to warm up.
- Once BWTS is warmed up and online it will automatically open its outlet valve, allowing treated ballast water to recirculate to the treatment pump.
- Close valves L and K.
- Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Place Water Quality Monitors online.
- Alert sampling team "Treatment Recirculation" via BOT. The sampling team will set flow rates. If required, due to rust in the line, the sampling team will alert via radio to Discharge to flush system.
 - Open valve R and close valve P, await signal from sampling team.
 - Once the system is flushed, the sampling team will alert via radio to return to recirculation. Open valve P and close valve R.
- The sampling team will prepare the system. Once the sampling system is prepared, the sampling team will return "Ready to Sample" via BOT. Sampling team tasks:
 - Flow sample water into sample tubs, but not through plankton nets.
 - Fill the sample tubs to the appropriate level.
 - Start water quality probes in sample tubs.
- Log piping system status (ballast treatment pump, BWTS, valves, bleeds).



















Test Cycle Number

PIC Initials

Quality Initials

Date

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")			(Open Valves Mark "O")			(Pull Plug, Open Valve Mark "OUT")			
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-2
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 14, Step 11 Treatment Tank 2 Discharge

- Ensure Facility is ready for discharge:
 - Facility piping system and BWTS is operating within valid test requirements.
 - Sampling team is “Ready to Sample” via BOT.
- Send discharge to overboard. Open valve R and close valve N.
- Alert sampling team “Treatment 2 Discharge” via BOT. Sampling team tasks:
 - Flow sample water through designated plankton nets.
 - Monitor water quality probes in sample tubs.
- Throttle valve G and use pump’s flow control mode to maintain 250 m3/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data. **Monitor and log filter backwash pressure.**

Once Treatment Tank 2 (3-154-2) has reached a level of ~300 mm (1 ft), the flow rate is reduced until tank is empty. At loss of suction, the BWTS is secured, and the pumping system is secured.

- Slow pumping rate to 50 m3/hr when Treatment Tank 2 (3-154-2) innage is at 300 mm (1 ft).
- When ballast treatment pump loses suction, secure the BWTS and treatment pump.
 - Select BWTS Stop through automation system interface.
 - Stop treatment pump and immediately close valve R to overboard. This ensures that the BWTS remains flooded during its shutdown cycle.
 - Secure all remaining valves left open.
- Confirm that the BWTS has completed its shut-down cycle.

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN")	
Pump				Device (BWTS)				(Pull Plug, Open Valve Mark "OUT")	
A	B	C		D	E	F1	F2	G	
Pump Manifold				Utility Manifold					
N	P	R		H	J	K	L	M	
Source Tanks				Treat. Tanks		Control			
ZV	ZX	W	ZT	T	V	ZW			
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct		
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY		
Ballast Main Piping							Ovbd		
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U		
								Bleed Plug & Valve	Location Status
								Seachest	A-B
								Source Tanks	W-ZT
								Treatment 1 Tank	J-P-T
								Treatment 2 Tank	H-N-V
								Device By-pass	F1-F2
								Fire/Ballast Pump	ZF-ZG
								Ballast Main - Pump	ZP-ZR
								Ballast Main - Tank	ZK-ZL
								Ballast Main - Tank	ZM-ZN

Test Cycle Number _____

PIC Initials _____

Quality Initials _____

Date _____

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-2
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

B/W Press (cycle)

#1

#2

#3

#4

#5


#6

Notes: _____

SOP 14, Step 12 Control Tank Discharge Recirculation

*****Both Treatment Tanks must be discharged BEFORE Control Tank*****

- Align valves and bleeds as per recirculation diagram. Throttle valve G to three (3) threads showing.
- Confirm 01 Deck by-pass is open and BWTS isolation valves are shut.
- Start Ballast Treatment Pump at low RPM. Bleed air from system at BWTS inlet vent.
- Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Alert sampling team "Treatment Recirculation" via BOT. The sampling team will set flow rates. If required, due to rust in the line, the sampling team will alert via radio to Discharge to flush system.
 - Open valve R and close valve N, await signal from sampling team.
 - Once the system is flushed, the sampling team will alert via radio to return to recirculation. Open valve N and close valve R.
- The sampling team will prepare the system. Once the sampling system is prepared, the sampling team will return "Ready to Sample" via BOT. Sampling team tasks:
 - Flow sample water into sample tubs, but not through plankton nets.
 - Fill the sample tubs to the appropriate level.
 - Start water quality probes in sample tubs.
- Log piping system status (ballast treatment pump, BWTS, valves, bleeds).

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN") (Pull Plug, Open Valve Mark "OUT")		
Pump				Device (BWTS)				Bleed Plug & Valve	Location	Status
A	B	C		D	E	F1	F2	G	Seachest	A-B
Pump Manifold				Utility Manifold					Source Tanks	W-ZT
N	P	R		H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks				Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW			Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct		Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY		Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd		Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U		Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials

Date

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 6-E-0
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						


Notes: _____

SOP 14, Step 13 Control Tank Discharge

- Ensure Facility is ready for discharge:
 - Facility piping system is operating within valid test requirements.
 - Sampling team is “Ready to Sample” via BOT.
- Send discharge to overboard. Open valve R and close valve N.
- Alert sampling team “Control Discharge” via BOT. Sampling team tasks:
 - Flow sample water through designated plankton nets.
 - Monitor water quality probes in sample tubs.
- Throttle valve G and use pump’s flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data.

Once Control Tank (6-E-0) has reached a level of ~300 mm (1 ft), the flow rate is reduced until tank is empty. At loss of suction, the pumping system is secured.

- Slow pumping rate to 50 m³/hr when Control Tank (6-E-0) innage is at 300 mm (1 ft).
- When ballast treatment pump loses suction, secure the treatment pump.
 - Stop treatment pump.
 - Secure all remaining valves left open.

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")			(Open Valves Mark "O")					(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZO	ZR	U	Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials

Date

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 6-E-0
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 15 Operation of BWTS and Facility Piping System (SHIPBOARD)

Application	LAND-BASED		SHIPBOARD	XX
-------------	------------	--	-----------	----

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Test Cycle Piping Diagrams identify system process points, instrumentation locations, and designate valve names. They are available in the automation system, on Facility laminated placards, and in key documents.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

Uptake Background Information

Ballast water uptake consists of one event that uptakes to the Treatment Tank (3-154-1) and then immediately uptakes to the Control Tank (3-154-2).

The BWTS inlet piping is primed, and water is pumped through a recirculation loop while the BWTS warms up. When the BWTS is ready, water is pumped to overboard (sea-to-sea). The sea-to-sea mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge from overboard to fill the Treatment Tank.

When the Treatment Tank reaches its designated volume, the team switches to start fill of the Control Tank while shutting down the BWTS. When the Control Tank reaches its designated volume, pumping is stopped to clean the piping system of control ballast water.

Communications between the pumping team (opening and closing valves, operating the treatment system pump, and operating the BWTS) and the sampling team is performed by radio, visual communications, and the automation system ballast order telegraph (BOT). Of these, the BOT is the primary and definitive means of communication.

The sampling team will prepare the sampling system by flushing all equipment, hoses, and other devices with raw seawater before uptake or held water before discharge. During set-up, the sampling team will also balance flows into the tubs where the plankton nets are rigged. However, the sampling team will only send ballast water through the plankton nets when directed by the pumping team. This is called “netting.” Only during an actual uptake or discharge are the samples netted, in order to maximize the sample representativeness of the ballast tank contents.

Please reference the Shipboard Piping Diagrams in SOP 3.

SOP 15, Step 1 Pipe Flushing

Prior to uptakes events, the Facility piping system is flushed with bleach solution. Flush pump loop, 01 deck loop, Tank 3-154-1, and Tank 3-154-2 branch lines.

Pipe Flushing Set-Up

- Secure tank bleeds at Valves V, T, and W.
- Connect and open “City Water” to pump suction.
- Connect bleach tank to pump suction. Add one (1) gallon of 5% bleach solution and mix with fresh water.
- Open pump loop Valves C, K, and M. Throttle L.
- Open D, E, F1, F2, and 1-1/2 inch vent valve at 01 Deck and vent air to fill system.
Note: May require periodic venting during flush cycles.
- Throttle valve L to create a vacuum at pump suction and draw bleach solution into piping system.

Pump Loop Flushing

- Start pump at 800 RPM. Using valve L as per above, draw and flush bleach solution into system. Vary throttle valve L and pump speed to maintain ~200 m³/hr of flow through the pump. Run minimum of five minutes.
- Log valve positions and log “Pump Loop Flushed.”

01 Deck Loop and Tank Branch Line Flushing

- Open valves P and J.
- Open throttle valve G to 2 threads. Close throttle valve L, while adjusting valve G and pump speed to maintain 150-200 m³/hr flow rate in Tank 3-154-1 branch. Run minimum of five minutes.
- Log valve positions and “01 Deck Loop Flushed” and “3-154-1 Branch Flushed.”
- Open control tank valves H and N. Close valves P and J. Run minimum of five minutes.
- Log valve positions and “3-154-2 Branch Flushed.”

Secure System

- Secure pump, city water, and open all bleeds.
- Drain all Facility piping through low-point drains using vents and system valves.
- Secure all valves. Log “Facility Piping Drained.”

Test Cycle Number

PIC Initials

Quality Initials

Date

Pump Loop Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V		ZW	Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

01 Deck and 3-154-1 Branch Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V		ZW	Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

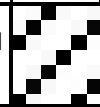
01 Deck and 3-154-2 Branch Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G		
Pump Manifold			Utility Manifold						
N	P	R	H	J	K	L	M	Seachest	A-B
Source Tanks			Treat. Tanks		Control			Source Tanks	W-ZT
ZV	ZX	W	ZT	T	V	ZW		Treatment 1 Tank	J-P-T
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Treatment 2 Tank	H-N-V
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Device By-pass	F1-F2
Ballast Main Piping							Ovbd	Fire/Ballast Pump	ZF-ZG
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Pump	ZP-ZR
								Ballast Main - Tank	ZK-ZL
								Ballast Main - Tank	ZM-ZN

Notes: _____

SOP 15, Step 2 Uptake Sea-to-Sea Mode

- Confirm Tanks 3-154-1 and 3-154-2 are empty and clean.
- Align valves and bleeds as per diagram. Keep valves E and R closed, and throttle valve G to three (3) threads showing.
- Confirm 01 Deck by-pass is double blocked and bled.
- Open valves L, K, M, and ZY to provide pump recirculation flow until BWTS is running.
- Start Ballast Treatment Pump at low RPM. Throttle valve L and bleed air from system at BWTS inlet vent.
- Turn on BWTS, set to Ballast Mode.
- Once BWTS is warmed up and online it will automatically open its outlet valve. Slowly open valve E and bleed air. Once line is charged, slowly open valve R, allowing treated ballast water to flow through discharge piping to the overboard.
- Close valves L, K, and M.
- Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Place Water Quality Monitors online.
- Alert sampling team "Uptake Sea to Sea" via Ballast Order Telegraph (BOT). The sampling team will prepare for sampling. Once the system is flushed, the sample team will return "Ready to Sample" via BOT. Sampling team tasks:
 - Flush sampling system with raw seawater (from inlet to BWTS system).
 - Flow sample water into sample tub(s), but not through plankton nets.
 - Start water quality probe(s) in sample tub(s).
- Log piping system status (ballast treatment pump, BWTS, valves, and bleeds).

(Shut Valves Mark "X") (Open Valves Mark "O") (Installed Plug, Shut Valve Mark "IN") (Pull Plug, Open Valve Mark "OUT")									
Pump				Device (BWTS)				Bleed Plug & Valve	Status
A	B	C		D	E	F1	F2	G	Seachest
Pump Manifold				Utility Manifold					Source Tanks
N	P	R		H	J	K	L	M	Treatment 1 Tank
Source Tanks				Treat. Tanks		Control		Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZO	ZR	U	Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials


Date

SYSTEM PARAMETERS			TIME Use 24 HR (#####)				
Position	#1	#2	#4	#5	#6	Tank 3-154-1	Tank 3-154-2
Flow (m3/hr)							
Temp (C)							
Press (kPa)							
Volume (m3)							

Notes: _____

SOP 15, Step 3 Treatment Uptake

- Ensure Facility is ready for uptake:
 - Facility piping system and BWTS is operating within valid test requirements.
 - Sampling team is “Ready to Sample” via BOT.
- Send discharge to Treatment Tank. Open valves P and T to Tank 3-154-1, then close valves R and U to overboard.
- Alert sampling team “Treatment Uptake” via BOT. Sampling team tasks:
 - Flow sample water through designated plankton nets.
 - Monitor water quality probe(s) in sample tub(s).
- Throttle valve G and use pump’s flow control mode to maintain 250 m3/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data.
- (See next step for switching system once tank has reached at least 200 m3 volume.)

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")			(Open Valves Mark "O")					(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

SYSTEM PARAMETERS			TIME Use 24 HR (####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-1
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes:


SOP 15, Step 4 Control Uptake

Once the Treatment Tank (3-154-1) has been filled to a volume not-less-than 200 m³, the system is switched to fill the Control Tank (3-154-2) and the BWTS is secured.

- Ensure Facility is ready for Control Uptake:
 - Treatment Tank 1 (3-154-1) volume is not-less-than 200 m³.
 - Facility piping system and BWTS is operating within valid test requirements.
 - Sampling team is "Sampling" via BOT.
- Send discharge to Control Tank.
 - Open valves N and V, then close valves P and T to begin filling Tank 3-154-2 and stop filling Tank 3-154-1.
 - Immediately open plugged drain between valves P and T at tank pipe inlet – double block and bleed.
- Secure BWTS.
 - Open 01 Deck by-pass valves F1 and F2.
 - Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
 - Select BWTS Stop through automation system interface.
 - Once the BWTS system is shutdown, isolate by closing valves D and E.
 - Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Alert sampling team "Control Uptake" via BOT. Sampling continues without change.
- Log Valve and System Data.

Once Control Tank (3-154-2) has been filled to a volume not-less-than 200 m³, the pumping system is secured.

- Stop treatment pump.
- Flow automatically stops at the sampling system.
- Secure all remaining valves left open.

								(Installed Plug, Shut Valve Mark "IN")			
(Shut Valves Mark "X")			(Open Valves Mark "O")					(Pull Plug, Open Valve Mark "OUT")			
Pump			Device (BWTS)					Bleed Plug & Valve		Location	Status
A	B	C	D	E	F1	F2	G	Seachest		A-B	
Pump Manifold			Utility Manifold					Source Tanks		W-ZT	
N	P	R	H	J	K	L	M	Treatment 1 Tank		J-P-T	
Source Tanks				Treat. Tanks		Control		Treatment 2 Tank		H-N-V	
ZV	ZX	W	ZT	T	V	ZW		Device By-pass		F1-F2	
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump		ZF-ZG	
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump		ZP-ZR	
Ballast Main Piping							Ovbd	Ballast Main - Tank		ZK-ZL	
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank		ZM-ZN	

Test Cycle Number

PIC Initials

Quality Initials

Date

SYSTEM PARAMETERS			TIME Use 24 HR (####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-2
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes:

SOP 15, Step 5 Tank Holding and Pipe Flushing

Treatment and Control tanks are held for not-less-than 48 hours.

After filling and before discharging, the Facility piping system is flushed with bleach solution. Flush pump loop, 01 deck loop, Tank 3-154-1, and Tank 3-154-2 branch lines.

Tank Holding and Monitoring

Once the uptake events are completed, perform the following.

- Ensure Facility piping system is secured.
 - Sound tanks to confirm volumes.
 - Ensure security of ballast treatment pump, BWTS, and all valves.
 - Ensure all valves isolating Treatment Tank (1-54-1) and Control Tank (1-154-2) are isolated by double block and bleed.
 - Log condition of piping system (pump, BWTS, valves and bleeds).
- Monitor tanks during holding period.
 - Treatment Tank (1-154-1) and Control Tank (3-154-2) for not-less-than 48 hours (Start to Start). This means if Treatment Uptake started on Monday at 0800 hours, then Treatment Tank discharge is to start no sooner than Wednesday at 0800 hours.
 - Log tank data every 12 hours.

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN")	
Pump				Device (BWTS)				(Pull Plug, Open Valve Mark "OUT")	
A	B	C		D	E	F1	F2	G	
Pump Manifold				Utility Manifold					
N	P	R		H	J	K	L	M	
Source Tanks				Treat. Tanks		Control			
ZV	ZX	W	ZT	T	V	ZW			
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct		
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY		
Ballast Main Piping							Ovbd		
ZJ	ZK	ZL	ZM	ZN	ZO	ZR	U		
								Bleed Plug & Valve	Location Status
								Seachest	A-B
								Source Tanks	W-ZT
								Treatment 1 Tank	J-P-T
								Treatment 2 Tank	H-N-V
								Device By-pass	F1-F2
								Fire/Ballast Pump	ZF-ZG
								Ballast Main - Pump	ZP-ZR
								Ballast Main - Tank	ZK-ZL
								Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials

Date

Tank 3-154-1	Date/ Time	Temp (C)	Volume (m3)	Tank 3-154-2	Date/ Time	Temp (C)	Volume (m3)

Notes: _____

Pipe Flushing Set-Up

- Secure tank bleeds at Valves V, T, and W.
- Connect and open “City Water” to pump suction.
- Connect bleach tank to pump suction. Add one (1) gallon of 5% bleach solution and mix with fresh water.
- Open pump loop Valves C, K, and M. Throttle L.
- Open D, E, F1, F2, and 1-1/2 inch vent valve at 01 Deck and vent air to fill system. Note: May require periodic venting during flush cycles.
- Throttle valve L to create a vacuum at pump suction and draw bleach solution into piping system.

Pump Loop Flushing

- Start pump at 800 RPM. Using valve L as per above, draw and flush bleach solution into system. Vary throttle valve L and pump speed to maintain ~200 m³/hr of flow through the pump. Run minimum of five minutes.
- Log valve positions and log “Pump Loop Flushed.”

01 Deck Loop and Tank Branch Line Flushing

- Open valves P and J.
- Open throttle valve G to 2 threads. Close throttle valve L, while adjusting valve G and pump speed to maintain 150-200 m³/hr flow rate in Tank 3-154-1 branch. Run minimum of five minutes.
- Log valve positions and “01 Deck Loop Flushed” and “3-154-1 Branch Flushed.”
- Open control tank valves H and N. Close valves P and J. Run minimum of five minutes.
- Log valve positions and “3-154-2 Branch Flushed.”

Secure System

- Secure pump, city water, and open all bleeds.
- Drain all Facility piping through low-point drains using vents and system valves.
- Secure all valves. Log “Facility Piping Drained.”

Test Cycle Number

PIC Initials

Quality Initials

Date

Pump Loop Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V		ZW	Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

01 Deck and 3-154-1 Branch Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Treatment 2 Tank	H-N-V	
ZV	ZX	W	ZT	T	V		ZW	Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

01 Deck and 3-154-2 Branch Flushing Log

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")				(Open Valves Mark "O")				(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Location Status
A	B	C	D	E	F1	F2	G		
Pump Manifold			Utility Manifold						
N	P	R	H	J	K	L	M	Seachest	A-B
Source Tanks			Treat. Tanks		Control			Source Tanks	W-ZT
ZV	ZX	W	ZT	T	V	ZW		Treatment 1 Tank	J-P-T
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Treatment 2 Tank	H-N-V
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Device By-pass	F1-F2
Ballast Main Piping							Ovbd	Fire/Ballast Pump	ZF-ZG
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Pump	ZP-ZR
								Ballast Main - Tank	ZK-ZL
								Ballast Main - Tank	ZM-ZN

Notes: _____

Recirculation and Discharge Background Information

Ballast water discharge proceeds following a holding time of not-less-than 48 hours. Discharge includes two events that discharge Treatment Tank (3-154-1) and Control Tank (3-154-2).

The first event primes the BWTS inlet piping, starts the BWTS, and takes suction from the Treatment Tank. The treated water passes the BWTS, and returns to the treatment pump suction (recirculation). The recirculation mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge to overboard, emptying the Treatment Tank to sea, while the sampling team takes triplicate sampling of the treated water. **The second event must follow the discharge of the Treatment Tank.** The Control Tank is recirculated, now by-passing the BWTS. The untreated control water is then sampled and discharged to sea.

Communication between the pumping team and sampling team is performed as it was for uptake procedures, where ballast order telegraph (BOT) is the primary and definitive means of communication. Sampling procedures are similar to that of uptake, but require different volumes of triplicate sampling during each discharge event.

SOP 15, Step 6 Treatment Tank – Discharge Recirculation

*****The Treatment Tank must be discharged BEFORE Control Tank*****

- Confirm Tanks 3-154-1 and 3-154-2 have been held for not-less-than 48 hours.
- Align valves and bleeds as per recirculation diagram. Throttle valve G to three (3) threads showing.
- Confirm 01 Deck by-pass is double blocked and bled.
- Open valves L and K to provide pump recirculation flow until BWTS is running.
- Start Ballast Treatment Pump at low RPM. Throttle valve L and bleed air from system at BWTS inlet vent.
- Turn on BWTS, set to De-Ballast Mode. Allow BWTS to warm up.
- Once BWTS is warmed up and online it will automatically open its outlet valve, allowing treated ballast water to recirculate to the treatment pump.
- Close valves L and K.
- Throttle valve G and use pump's flow control mode to maintain 250 m3/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Place Water Quality Monitors online.
- Alert sampling team "Treatment Recirculation" via BOT. The sampling team will set flow rates, and alert via radio to Discharge to flush system.
- Open valve R and close valve P, await signal from sampling team.
- Once the system is flushed, the sampling team will alert via radio to return to recirculation. Open valve P and close valve R.
- The sampling team will prepare the system. Once the sampling system is prepared, the sampling team will return "Ready to Sample" via BOT. Sampling team tasks:
 - Flow sample water into sample tubs, but not through plankton nets.
 - Fill the sample tubs to the appropriate level.
 - Start water quality probes in sample tubs.
- Log piping system status (ballast treatment pump, BWTS, valves, bleeds).

Operator has option to filter on discharge with filter backwash directed to ballast tank.

Test Cycle Number

PIC Initials

Quality Initials

Date

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")			(Open Valves Mark "O")			(Pull Plug, Open Valve Mark "OUT")			
Pump			Device (BWTS)			Bleed Plug & Valve		Location	Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold			Source Tanks		W-ZT	
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control	Treatment 2 Tank		H-N-V	
ZV	ZX	W	ZT	T	V	ZW	Device By-pass	F1-F2	
Skin Valves		Drain Valves		Fire/Ballast Pump		Suct	Fire/Ballast Pump	ZF-ZG	
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping						Ovbd	Ballast Main - Tank	ZK-ZL	
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U	Ballast Main - Tank	ZM-ZN

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-1
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 15, Step 7 Treatment Tank Discharge

- Ensure Facility is ready for discharge:
 - Facility piping system and BWTS is operating within valid test requirements.
 - Sampling team is “Ready to Sample” via BOT.
- Send discharge to overboard. Open valves R and U, and close valve P.
- Alert sampling team “Treatment Discharge” via BOT. Sampling team tasks:
 - Flow sample water through designated plankton nets.
 - Monitor water quality probes in sample tubs.
- Throttle valve G and use pump’s flow control mode to maintain 250 m3/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data.

Once the Treatment Tank (3-154-1) has reached a level of ~300 mm (1 ft), the flow rate is reduced until tank is empty. At loss of suction, the BWTS is secured, and the pumping system is secured.

- Slow pumping rate to 50 m3/hr when the Treatment Tank (3-154-1) innage is at 300 mm (1 ft).
- When ballast treatment pump loses suction, secure the BWTS and treatment pump.
 - Select BWTS Stop through automation system interface.
 - Stop treatment pump and immediately close valve R to overboard. This ensures that the BWTS remains flooded during its shutdown cycle.
 - Secure all remaining valves left open.

Confirm that the BWTS has completed its shut-down cycle.

(Shut Valves Mark "X")			(Open Valves Mark "O")			(Pull Plug, Open Valve Mark "OUT")			(Installed Plug, Shut Valve Mark "IN")	
Pump			Device (BWTS)			Bleed Plug & Valve			Location	Status
A	B	C	D	E	F1	F2	G		Seachest	A-B
Pump Manifold			Utility Manifold						Source Tanks	W-ZT
N	P	R	H	J	K	L	M		Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks			Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW			Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct		Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY		Ballast Main - Pump	ZP-ZR
Ballast Main Piping						Ovbd			Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZP	ZR	U		Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials

Date

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-1
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 15, Step 8 Control Tank – Discharge Recirculation

- Align valves and bleeds as per recirculation diagram. Throttle valve G to three (3) threads showing.
- Confirm 01 Deck by-pass is open and BWTS isolation valves are shut.
- Start Ballast Treatment Pump at low RPM. Bleed air from system at BWTS inlet vent.
- Throttle valve G and use pump's flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Alert sampling team "Treatment Recirculation" via BOT. The sampling team will set flow rates, and alert via radio to Discharge to flush system.
- Open valve R and close valve P, await signal from sampling team.
- Once the system is flushed, the sampling team will alert via radio to return to recirculation. Open valve P and close valve R.
- The sampling team will prepare the system. Once the sampling system is prepared, the sampling team will return "Ready to Sample" via BOT. Sampling team tasks:
 - Flow sample water into sample tubs, but not through plankton nets.
 - Fill the sample tubs to the appropriate level.
 - Start water quality probes in sample tubs.
- Log piping system status (ballast treatment pump, BWTS, valves, bleeds).

(Shut Valves Mark "X") (Open Valves Mark "O")								(Installed Plug, Shut Valve Mark "IN")	
								(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control		Suct	Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Ovbd	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG		Ballast Main - Pump	ZP-ZR
Ballast Main Piping							U	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZO	ZR		Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials

Date

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-2
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						


Notes: _____

SOP 15, Step 9 Control Tank Discharge

- Ensure Facility is ready for discharge:
 - Facility piping system is operating within valid test requirements.
 - Sampling team is “Ready to Sample” via BOT.
- Send discharge to overboard. Open valve R and close valve N.
- Alert sampling team “Control Discharge” via BOT. Sampling team tasks:
 - Flow sample water through designated plankton nets.
 - Monitor water quality probes in sample tubs.
- Throttle valve G and use pump’s flow control mode to maintain 250 m³/hr +/- 10% and ~1.0 bar back pressure at Position 5.
- Log Valve and System Data.

Once Control Tank (3-154-2) has reached a level of ~300 mm (1 ft), the flow rate is reduced until tank is empty. At loss of suction, the pumping system is secured.

- Slow pumping rate to 50 m³/hr when Control Tank (6-E-0) innage is at 300 mm (1 ft).
- When ballast treatment pump loses suction, secure the treatment pump.
 - Stop treatment pump.
 - Secure all remaining valves left open.

								(Installed Plug, Shut Valve Mark "IN")	
(Shut Valves Mark "X")			(Open Valves Mark "O")					(Pull Plug, Open Valve Mark "OUT")	
Pump			Device (BWTS)					Bleed Plug & Valve	Status
A	B	C	D	E	F1	F2	G	Seachest	A-B
Pump Manifold			Utility Manifold					Source Tanks	W-ZT
N	P	R	H	J	K	L	M	Treatment 1 Tank	J-P-T
Source Tanks			Treat. Tanks		Control			Treatment 2 Tank	H-N-V
ZV	ZX	W	ZT	T	V	ZW		Device By-pass	F1-F2
Skin Valves		Drain Valves		Fire/Ballast Pump			Suct	Fire/Ballast Pump	ZF-ZG
ZA	ZB	ZC	ZD	ZE	ZF	ZG	ZY	Ballast Main - Pump	ZP-ZR
Ballast Main Piping							Ovbd	Ballast Main - Tank	ZK-ZL
ZJ	ZK	ZL	ZM	ZN	ZO	ZR	U	Ballast Main - Tank	ZM-ZN

Test Cycle Number

PIC Initials

Quality Initials

Date

SYSTEM PARAMETERS			TIME Use 24 HR (#####)			
Position	#1	#2	#4	#5	#6	Tank 3-154-2
Flow (m3/hr)						
Temp (C)						
Press (kPa)						
Volume (m3)						

Notes: _____

SOP 16 Operation of Ballast Water Sampling System (LAND-BASED)

Application	LAND-BASED	XX	SHIPBOARD	
-------------	------------	----	-----------	--

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Test Cycle Piping Diagrams identify system process points, instrumentation locations, and designate valve names. They are available in the automation system, on Facility laminated placards, and in key documents.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

Ballast Water Sampling System Operations

These procedures are performed to provide samples to support the analyses described in the Scientific Assays. The below table, Sampling Procedure Coordination, matches the pumping system operation to a particular sampling procedure and the resulting sample replicates and volumes.

Sampling Procedure Coordination Table

Pumping Operations		Sampling Procedures					
	Rate** (m3/hr)	Name	Location	Pitot/ Size (inches)	Rate** (lpm)	Each Replicate	
						Volume	
						10 - 50 µm	≥50 µm
Uptake							
Treatment 1 Untreated	250	3 x 0.4	B1, B2, B3	S1 (1 x 1.05")	8	3 x 22 liters	3 x 0.4m3
Treatment 1 Treated	250	T-0	170L	S2 (1 x 0.58")	drip	170L	
Treatment 2 Untreated	250	3 x 0.4	B1, B2, B3	S1 (1 x 1.05")	8	3 x 22 liters	3 x 0.4m3
Treatment 2 Treated	250	T-0	170L	S2 (1 x 0.58")	drip	170L	
Control (Untreated)	250	3 x 0.4	A1, A2, A3	S1 (1 x 1.05")	8	3 x 22 liters	3 x 0.4m3
Discharge							
Treatment 1 Treated	250	3 x 3	A1, A2, A3	S4 (3 x 1.68")	75	3 x 22 liters	3 x 3m3
Treatment 2 Treated	250	3 x 3	A1, A2, A3	S4 (3 x 1.68")	75	3 x 22 liters	3 x 3m3
Control (Untreated)	250	3 x 1	B1, B2, B3	S2 (1 x 1.61")	25	3 x 22 liters	3 x 1m3

**During tank stripping pumping rates slows down.

Uptake sample
flow is 22.3 lpm

Treatment 1, Treatment 2,
then Control

Sampling Overview

Ballast water sampling procedures vary as per the ballasting operations as follows:

Ballast Water Uptake Untreated Water Samples (9 x 0.4 m³ and 9 x 22 liters).

During ballast water uptake, untreated water is taken from the Source Tanks and moved to Treatment Tank 1, ~~then Control Tank, and then to Treatment Tank 2.~~ At each of these three steps, three untreated sample of at least 0.4 cubic meters is taken before the BWTS. This method assures sampling during the entire uptake event, and produces three (3) triplicate samples for each uptake totaling in greater than one (1) cubic meter. From each uptake, a 22-88L carboy sample (based on organism density) will be taken from each 0.4 m³ sample, and each will be filtered for analysis of the ≥ 50 μ m size class.

During each uptake a carboy of 22 liters is filled continuously, unfiltered, from each sample tub. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 μ m size class, for each uptake. A total of nine (9) 20 L samples for the 10 – 50 μ m size class are collected.

Ballast Water Uptake Treated Water Samples (2 x 170 liters).

During ballast water uptake to Treatment Tank 1 and Treatment Tank 2, 170 liters of treated water is sampled after the BWTS. The 170L sample is split into one-third volumes at the beginning, middle, and end of each uptake. This provides three (3) samples totaling in 170 liters for each uptake. **Ballast Water Discharge Treatment Tank 1 Samples (3 x 3m³ and 3 x 22 liters).**

During ballast water discharge from Treatment Tank 1, integrated samples of treated water in triplicate of at least 3.0 cubic meters are each taken after the BWTS. This provides a total of three (3) 3.0 cubic meter samples for a total volume of at least 9.0 cubic meters. This method assures sampling during the entire discharge event for analysis of the ≥ 50 μ m size class.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from the same sample line. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 μ m size class.

Ballast Water Discharge Treatment Tank 2 Samples (3 x 3m³ and 3 x 22 liters).


These procedures are identical to Discharge Treatment Tank 1 Samples, producing three (3) 3.0 cubic meter samples for a total volume of at least 9.0 cubic meters for analysis of the ≥ 50 μ m size class and three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 μ m size class.

Ballast Water Discharge Control Samples (3 x 1m³ and 3 x 22 liters).

During ballast water discharge from Control Tank, integrated samples of treated water are taken in triplicate of at least 1.0 cubic meters each. This provides a total of three (3) 1.0 cubic meter samples for a total volume of at least 3.0 cubic meters. This method assures sampling during the entire discharge event for analysis of the ≥ 50 μ m size class.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from the same sample line. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 μ m size class

Sampling Coordination

Ballast water uptake includes one event that uptakes to Treatment Tank 1 (~~3-154-1~~) and then the Control Tank (~~6-E-0~~), and a second event that uptakes to Treatment Tank 2 (3-154-2). This sequence allows the one control tank to serve both treatment tanks. then to Control. 

The first uptake event primes the BWTS inlet piping, starts the BWTS, and passes ballast water from the seachest through the BWTS and then overboard (sea-to-sea). The sea-to-sea mode enables the sampling team to set-up the sampling system. The pumping team then: switches suction to the Source Tanks, fills Treatment Tank 1 with treated water, and switches to start fill of the Control Tank while shutting down the BWTS. Pumping is then stopped to clean the piping system of control ballast water. After the brief stop for cleaning, Treatment Tank 2 is filled in the same manner as Treatment Tank 1.

Ballast water discharge proceeds after ballast water is held for not-less-than 120 hours. Discharge includes three events that discharge Treatment Tank 1 (3-154-1), Treatment Tank 2 (3-154-2), and Control Tank (6-E-0).

The first discharge event primes the BWTS inlet piping, starts the BWTS, and takes suction from Treatment Tank 1. The treated water passes the BWTS, and returns to the treatment pump suction (recirculation). The recirculation mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge to overboard, emptying Treatment Tank 1 to sea, while the sampling team takes triplicate samples of the treated water. In the second event, Treatment Tank 2 is discharged in the same manner as Treatment Tank 1. The Control Tank is then recirculated, by-passing the BWTS. The untreated control water is then sampled and discharged to sea.

Communications between the pumping team (opening and closing valves, operating the treatment system pump, and operating the BWTS) and the sampling team is performed by radio, visual communications, and the automation system ballast order telegraph (BOT). Of these, the BOT is the primary and definitive means of communication.

The sampling team will prepare the sampling system by flushing all equipment, hoses, and other devices with raw seawater before uptake or held ballasted before discharge. During set-up, the sampling system will also balance flows into the tubs where the plankton nets are rigged. However, the sampling team will only send ballast water through the plankton nets when directed by the pumping team. This is called “netting.” Only during an actual uptake or discharge are the samples netted, in order to maximize the sample representativeness of the ballast tank contents.

Uptake Set-up

Procedure 3 x 0.4 Set-up

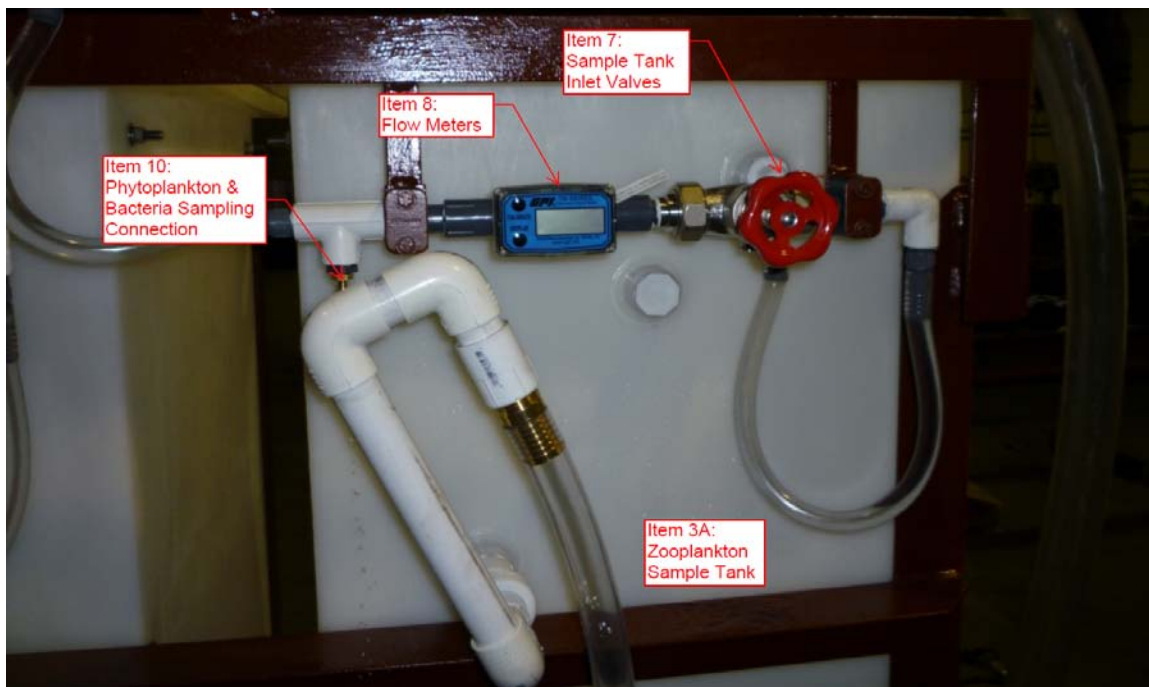
This step specifies critical equipment, pipe/hose connections, and system line-up required for 3 x 0.4 m3 sampling.

- Ensure the manifold inlet piping is flushed with chlorinated fresh water and drained. The Dump Valve to the drain tank (Item 9) should be closed.
- Use the sample port located on the Main Deck (P&ID Position S1), with the ~~3/4 in~~ 1.05" I.D. sampling pitot and flow meter installed.
- Use sample assembly tubs as designated above for each process. Align (but do not connect) sample port S1 to the Sample Manifold (Item 4) using 1-in Clear PVC Hose (Item 5).
- Prepare the six (6) Zooplankton Sample Tubs (Items 1, 2, & 3).
 - Each tub should be rinsed with fresh water and drained.
 - Inspect internal and external tubing and piping to ensure connection to the sample manifold and drain tank.
 - The Sample Tub Inlet Valves (Item 7) should be SHUT. The Tub Dump Valves (Item 6) should be SHUT, allowing the weir pipe to engage.
 - Nets should NOT be installed in any of the tubs.
- Ensure the MagFlo flow meter is operational.
- Connect Drain Tank Sump Pump (Item 13) to power, and connect Sump Pump “Dump to Overboard” line to a waste connection.

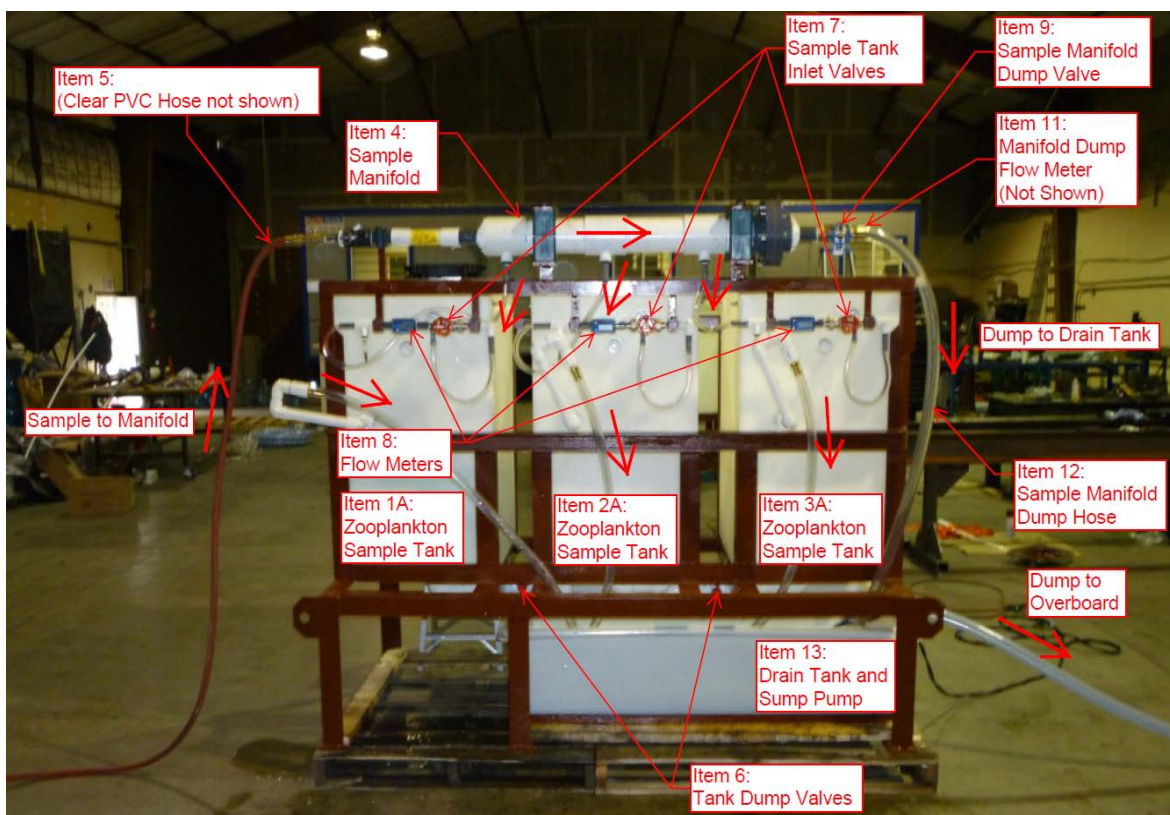
Procedure T0 Set-up

Connect sample port S2 for filling a 170L container using a Clear PVC Hose. Disconnect for flushing purposes. *This sample is not taken during Control uptake or any discharge processes.*

Notes: _____



Sample Tank Inlet Pipe Construction (Typ.)



Sampling Assembly (Assembly "A" Shown)

SOP 16, Step 1 Uptake Sampling Flushing

Uptake sampling is taken by filling THREE tubs for each of the following: Treatment 1 Uptake, Control Uptake, and Treatment 2 Uptake. The following prepares the tubs.

Sample Pitot Isokinetic Flow Rates – Uptake Mode

Flow Rates for 1.1m3 Sample w/ 1.05 Inch I.D. Pitot at 48 Minutes				
Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate/ MagFlo Sensor	1.2	5.6	5.9	6.1

- Lead Operator will indicate on BOT “Sea-to-Sea.” This indicates that the sampling system can now be flushed and prepared.
- Flush sample port S1 and sample port S2 hoses over the side for not less than one minute, and until relatively clear water is achieved.
- Connect sample port S1 to Sample Tub Manifold. Open the Sample Manifold Dump Valve (Item 9). Ensure the Sump Pump and Float Switch assembly (Item 13) is operational.
- Open the Sample Tank Inlet Valves (Item 7) for B1, B2, and B3 and adjust weir pipes to ensure the following:
 - Water should flow into the tubs.
 - In-tub hosing should be arranged in each tub to gently fill from the bottom of the tub.
 - The tub dump valves should be open, allowing water to leave the tubs.
- Prepare the first tubs (B1, B2, B3) for zooplankton collection.
 - Adjust flow through MagFlo sensor accordingly to the flow rate chart (above).
 - Reset flow meter totalizer.
 - Make sure other tubs are also prepared for sampling, but there should be no flow at this time.
 - Close inlet valve to B1, B2, and B3, and close tub dump valves.
- Prepare the 170L container for post-treatment sampling.
- Using BOT report to Lead Operator “Ready to Sample.”

SOP 16, Step 2 Uptake Sampling

The BWTS will have already been placed online during the sea-to-sea operation. Sampling begins immediately after uptake actually starts and finishes as each tank is filled.

Zooplankton Sampling

- The sampling team, as per previous step, should be in a position of “Ready to Sample” and indicate this on the BOT. The pumping team will be indicating “Sea-to-Sea” on the BOT.
- The Lead Operator will signal via the BOT when to start sampling as follows:
 - Treatment Tank 1 Uptake – Use Tubs B1, B2, B3
 - Treatment Tank 2 Uptake – Use Tub ~~B1, B2, B3~~ A1, A2, A3
 - Control Tank Uptake – Use Tub ~~A1, A2, A3~~ B1, B2, B3
- The sampling team will immediately open the three tub inlet valves and start sampling at these BOT signals, log the sampling into the automation system, and use the BOT to match the appropriate pumping team signal, e.g. Treatment Tank 1 Uptake.
- Each tub will fill completely during the course of the uptake.
- Monitor each tank level’s hash marks throughout the uptake.
- If tank levels become uneven, rebalance the tanks by throttling the tank inlet valves. At least one inlet valve should always be completely open.
- **Note:** During the Control Tank Uptake, the pumping team will switch over from Source Tank 1 to Source Tank 2.
- With approximately 1 foot remaining in tank, Lead Operator will open Source Tank 2 suction valve, close Source Tank 1 suction valve, making all efforts to maintain required flow rate.
- When Lead Operator indicates that an uptake event has been completed, stop sampling in those tubs by closing the inlet valves, and log event.
- When Lead Operator indicates that the “next” uptake event has begun, start sampling in the next set of tubs by opening the inlet valves (see *Sampling Procedure Coordination Table*), and log event.

Phytoplankton and Bacteria Sampling

- Use small tubing lines attached to the Phytoplankton & Bacteria Sampling Connection (Item 10) to gain samples for phytoplankton and bacteria.
- Gain three continuous samples for each event for a total of nine (9) continuous samples. Each of these samples should cover the entire ballasting event, either as a periodic fill such that eventually the full sample is obtained, or as a continuous drip feed.
- Log sampling times for each of the Phytoplankton samples. Label each collected sample.

After Treatment Uptake Sampling

- Sampling of treated uptake water only occurs during the two treatment uptake cycles.
- Move 1-inch Clear PVC Hoses attached to the Sample Port S2 pipe to the designated 170L container.
- Gain a total of three (3) continuous samples for each treatment tank fill event totaling in 170L: one at the beginning, one at the middle, and one at the end.
- Log sampling times for each of the three (3) Treated Uptake samples. Label each collected sample.

SOP 16, Step 3 Uptake Stopping and Sample Handling

Stop and secure the ballast sampling system, and process the samples as indicated below.

Stopping Sampling

- On uptake, sampling shall be stopped at completion of cycle by direction of the Lead Operator.
- Close the Sample Manifold Dump Valve (Item 9) and local valve at Pitot Tube (see Sample Assembly Construction figure, and allow the Sump Pump to empty the Drain Tank (Item 13) automatically.
- Disconnect the 1-inch PVC hose from the Sample Manifold (Item 4) to avoid contamination.
- Stir the tubs to homogenize the samples. Take a 22-88L carboy sample from each of the three tubs for zooplankton analysis. Lead Scientist is to select appropriate volume based on the expected organism density of the tub contents.
- Concentrate a single zooplankton sample for immediate analysis.

Sample Processing

- Samples must be clearly labeled and immediately brought to the Biological Laboratory for processing.
- Sample handling and analysis protocols are provided in a separate document.

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

SOP 16, Step 4 Cleaning

After collecting the ballast samples, clean the system as soon as possible as indicated below to prevent fouling or organism growth in the system.

- Drain all tanks, hoses, manifolds;
- Immediately flush with mild fresh water and bleach solution;
- Drain all components;
- Discard any components which are fouled and not readily cleaned.

Notes: _____

Discharge Set-up**Procedure 3x3 Set-up (Prior to Discharge Operations)**

This step specifies critical equipment, pipe/hose connections, and system line-up required for 3x3 sampling.

- Ensure that tank inlet piping is flushed with chlorinated fresh water and drained. The Dump Valve to the drain tank (item 9) should be closed.
- Use the sample port located on the Main Deck (P&ID Position S4), with the triple 1-1/2 sampling pitot installed.
- Use sample assembly tubs as designated above for each process. Align (but do not connect) each pipe of sample port S4 to the inlet piping of each sample tub, using 1 inch clear PVC hose. The hose should be connected directly to the sample tub inlets, in way of the sampling manifold.
- Prepare the three (3) Zooplankton Sample Tubs (Items 1, 2, & 3).
 - Each tub should be rinsed with fresh water and drained.
 - Inspect internal and external tubing and piping to ensure connection to the sample tubing and drain tank.
 - The Sample Tub Inlet Valves (Item 7) should be SHUT. The Tub Dump Valves (Item 6) should be SHUT, allowing the weir pipe to engage.
 - Install the collection nets in each of the three Zooplankton Sample Tubs.
- Ensure all Flow Meters (Item 8) are operational.
- Connect Drain Tank Sump Pump (Item 13) to power, and connect Sump Pump “Dump to Overboard” line to a waste connection.

Procedure 3x1 Set-up (Prior to Discharge Operations)

This step specifies critical equipment, pipe-hose connections, and system line-up required for 3x1 sampling.

- Ensure the manifold inlet piping is flushed with chlorinated fresh water and drained. The Dump Valve to the drain tank (Item 9) should be closed.
- Use the sample port located on the Main Deck (P&ID Position S2), with the 1-1/2 sampling pitot installed.
- Use sample assembly tubs as designated above for each process. Align (but do not connect) sample port S2 to the Sample Manifold (Item 4) using 1-1/2 inch Clear PVC Hose (Item 5).
- Prepare the three (3) Zooplankton Sample Tubs (Items 1, 2, & 3).
 - Each tub should be rinsed with fresh water and drained.
 - Inspect internal and external tubing and piping to ensure connection to the sample manifold and drain tank.

- The Sample Tub Inlet Valves (Item 7) should be SHUT. The Tub Dump Valves (Item 6) should be SHUT, allowing the weir pipe to engage.
- Install the collection nets in each of the three Zooplankton Sample Tubs.
- Ensure all Flow Meters (Items 8 & 11) are operational.
- Connect Drain Tank Sump Pump (Item 13) to power, and connect Sump Pump “Dump to Overboard” line to a waste connection.

Notes: Set up to collect sample from filter backwash for zooplankton and phytoplankton analysis. Volume will depend on filter cycles.

SOP 16, Step 5 Discharge Flushing

Treatment Discharge Flushing

Treatment Discharge sampling is taken by filling THREE tubs for Treatment 1 Discharge, and THREE tubs for Treatment 2 Discharge, of 3 cubic meters each. The Treatment Discharges are sampled with the 3x3 procedure.

Sample Pitot Isokinetic Flow Rates – Treatment Discharge Mode

Flow Rates for 10.8m³ Sample w/ 3x1.68 Inch I.D. Pitots at 48 Minutes				
Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate per Pitot/ Sample to Tanks (gpm)	4.0	19.0	19.8	20.6
Throttle Flow Meters as per Table				
Tub 1 Rate (gpm)*	3.97	19.04	19.83	20.63
Tub 2 Rate (gpm)*	3.97	19.04	19.83	20.63
Tub 3 Rate (gpm)*	3.97	19.04	19.83	20.63

- Lead Operator will indicate on BOT “Treatment Recirculation.” This indicates that the sampling system can now be flushed and prepared.
- Flush sample port S4 hoses over the side for not less than one minute, and until relatively clear water is achieved.
- Connect sample port S4 hoses to the Sample Tank A inlets. Ensure the Sump Pump and Float Switch assembly (Item 13) is operational.
- Open each Sample Tub Inlet Valve (Item 7) for A1, A2, and A3, throttling to achieve tub flow rates to each sample tub as specified by the flow rate chart (above).
- Disconnect sample port hoses from the sampling tubs, and alert Lead Operator via radio to switch to Discharge to flush system. Sampling line is flushed prior to Treatment 2 Discharge only if necessary, due to rust in the line.
 - System will be discharged to overboard for not less than one minute, and until relatively clear water is achieved.
 - Once flushing is completed, reconnect sample port hoses and alert Lead Operator to switch back to recirculation.
- Shut the Tub Dump Valves (Item 6) and adjust weir pipes to fill the tubs, and ensure the following:
 - Keep net bodies out of the influent flow. Water should flow into the tubs.
 - Collection net bodies should be “flooded” with the sample water in the tubs.
 - Collection net tops should be above the top of the tub water level.

- Collection piping should be submerged about 2 inches from the net cod end, so that sample entry into the tubs is gentle.
- Collection net cod end should be about 3 inches from the tub bottom.
- Prepare tubs A1, A2, and A3 for zooplankton collection.
 - Adjust flow rates into each tub (but not through net) according to the flow rate chart (above).
 - Reset flow meter totalizer.
 - Monitor and balance flow in each tub as necessary.
 - Open tub dump valves as necessary to control tub level.
- Using BOT report to Lead Operator “Ready to Sample.”

Control Discharge Flushing

Control Discharge sampling is taken by filling THREE tubs of 1 cubic meter each. The Control Discharge is sampled with the 3x1 procedure.

Sample Pitot Isokinetic Flow Rates – Control Discharge Mode

Flow Rates for 3.6m ³ Sample w/ 1.61 Inch I.D. Pitot at 48 Minutes				
Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate/ Sample to Manifold (gpm)	4.0	19.1	19.9	20.7
Throttle Flow Meters as per Table				
Tub 1 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 2 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 3 Rate (gpm)*	1.33	6.37	6.64	6.90

- Lead Operator will indicate on BOT “Control Recirculation.” This indicates that the sampling system can now be flushed and prepared.
- Flush sample port S2 hose over the side for not less than one minute, and until relatively clear water is achieved.
- Connect sample port S2 to Sample Tub Manifold B. Ensure the Sump Pump and Float Switch assembly (Item 13) is operational.
- Open each Sample Tub Inlet Valve (Item 7) for B1, B2, and B3, throttling to achieve tub flow rates to each sample tub as specified by the flow rate chart (above).
- Disconnect sample port hose from manifold, and alert Lead Operator via radio to switch to Discharge to flush system. Sampling line is flushed prior to Control Discharge only if necessary, due to rust in the line.
 - System will be discharged to overboard for not less than one minute, and until relatively clear water is again achieved.

- Once flushing is completed, reconnect sample port hose and alert Lead Operator to switch back to recirculation.
- Shut the Tub Dump Valves (Item 6) and adjust weir pipes to fill the tubs, and ensure the following:
 - Keep net bodies out of the influent flow. Water should flow into the tubs.
 - Collection net bodies should be “flooded” with the sample water in the tubs.
 - Collection net tops should be above the top of the tub water level.
 - Collection piping should be submerged about 2 inches from the net cod end, so that sample entry into the tubs is gentle.
 - Collection net cod end should be about 3 inches from the tub bottom.
- Prepare tubs B1, B2, and B3 for zooplankton collection.
 - Adjust flow rates into each tub (but not through net) according to the flow rate chart (above).
 - Reset flow meter totalizer.
 - Monitor and balance flow in each tub as necessary.
- Using BOT report to Lead Operator “Ready to Sample.”

SOP 16, Step 6 Discharge Sampling

Sampling shall begin immediately after the treatment system is started and operating at steady state, and just before the ballast treatment recirculation mode has been secured, and test cycle is started. The sampling procedure shall be repeated sequentially for Treatment 1 Discharge, Treatment 2 Discharge, and Control Discharge.

Zooplankton Sampling

- The sampling team, as per previous step, should be in a position of “Ready to Sample” and indicate this on the BOT. The pumping team will be indicating “Recirculation” on the BOT.
- The Lead Operator will signal via the BOT when to start “netting” as follows:
 - Treatment Discharge – Use Tubs A1, A2, and A3
 - Control Discharge – Use Tubs B1, B2, and B3
- The sampling team will immediately start netting at these BOT signals, log the netting into the automation system, and use the BOT to match the appropriate pumping team signal, i.e. Treatment Discharge.
- If a sample net becomes overwhelmed with material, close the Sample Tank Inlet Valve (Item 7), remove sample net, replace with a new net, open the Sample Tank Inlet Valve, and log event.
- **Note:** At end of the each discharge cycle (Treatment 1, Treatment 2, Control), the system flow will be reduced and isokinetic sample flow will be decreased as follows.
 - With approximately 1 foot remaining in tank, Lead Operator will reduce flow in system to 50 m³/hr while maintaining 200-250 kPa at position 2. There will remain 10-15 minutes estimated discharge time and sampling will be adjusted accordingly.
 - Adjust the Sample Tub Inlet Valves (Item 7) to each of the three (3) Zooplankton Sample Tanks (Items 1, 2, & 3), throttling to maintain tank flow rates to each sample tub as specified by the Tub Rates in the Sample Pitot Isokinetic Flow Rates tables.
- When Lead Operator indicates that a discharge event has been completed, stop netting, close tub dump valves, and log event.
- Repeat the process for each of the three discharge events.

Phytoplankton and Bacteria Sampling

- Use small tubing lines attached to the Phytoplankton & Bacteria Sampling Connection (Item 10) to gain samples for phytoplankton and bacteria.
- Gain three (3) continuous samples for each discharge event, one from each sampling tub. Each of these samples should cover the entire ballasting event, either as a periodic fill such that eventually the full sample is obtained, or as a continuous drip feed.
- Log sampling times for each event’s three (3) Phytoplankton samples. Label each collected sample.

Collect sample from filter backwash for zooplankton and phytoplankton analysis. Volume will depend on filter cycles.

Test Cycle Number

PIC Initials

Quality Initials

Date

Notes: _____

Stopping and Sample Handling

Stop and secure the ballast sampling system, and process the samples as indicated below.

Stopping Sampling

- On discharge events, with 1 foot remaining in tank flow will be reduced to 50 m³/hr and sample flow will be reduced proportionally. When flow is lost approximately 10-15 minutes after this flow reduction, the sample inlet valves are immediately shut.
- Close the local valves at Pitot Tube, and allow the Sump Pump to empty the Drain Tank (Item 13) automatically.
- Disconnect the 1 inch PVC hoses from the Sample Tank A inlets and the 1-1/2 inch PVC hose from Sample Manifold B to avoid contamination.
- Rinse plankton nets by gently submerging and lifting in full sample tub. Examine net using microscopy to confirm “loss percentage” of organisms.

Sample Processing

- Samples must be clearly labeled and immediately brought to the Biological Laboratory for processing.
- Sample handling and analysis protocols are provided in a separate document.

Notes: _____

Cleaning

After collecting the ballast samples, clean the system as soon as possible as indicated below to prevent fouling or organism growth in the system.

- Drain all tanks, hoses, pipes;
- Immediately flush with mild fresh water and bleach solution;
- Drain all components;
- Discard any components which are fouled and not readily cleaned.

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Treatment 1 Uptake Sample Collection Logs

ZOOPLANKTON 3x 0.4 SAMPLE LOG: TREATMENT 1 UPTAKE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)		(time)	(date)

PHYTOPLANKTON 3x0.4 SAMPLE LOG: TREATMENT 1 UPTAKE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)		(time)	(date)

POST-TREATMENT 3X20L SAMPLE LOG: TREATMENT 1 UPTAKE			
Label Name			
Quantity (liters)			
Time			
(initial)		(time)	(date)

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Control Uptake Sample Collection Logs

ZOOPLANKTON 3x 0.4 SAMPLE LOG: CONTROL UPTAKE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial) (time) (date)			

PHYTOPLANKTON 3x0.4 SAMPLE LOG: CONTROL UPTAKE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial) (time) (date)			

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Treatment 2 Uptake Sample Collection Logs

ZOOPLANKTON 3x 0.4 SAMPLE LOG: TREATMENT 2 UPTAKE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)		(time)	(date)

PHYTOPLANKTON 3x0.4 SAMPLE LOG: TREATMENT 2 UPTAKE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)		(time)	(date)

POST-TREATMENT 3X20L SAMPLE LOG: TREATMENT 2 UPTAKE			
Label Name			
Quantity (liters)			
Time			
(initial)		(time)	(date)

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Treatment 1 Discharge Sample Collection Logs

ZOOPLANKTON 3x3 SAMPLE LOG: TREATMENT 1 DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)	(time)	(date)	

PHYTOPLANKTON 3x3 SAMPLE LOG: TREATMENT 1 DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)	(time)	(date)	

Notes: _____

Treatment 2 Discharge Sample Collection Logs

ZOOPLANKTON 3x3 SAMPLE LOG: TREATMENT 2 DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)	(time)	(date)	

PHYTOPLANKTON 3x3 SAMPLE LOG: TREATMENT 2 DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)	(time)	(date)	

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Control Discharge Sample Collection Logs

ZOOPLANKTON 3x1 SAMPLE LOG: CONTROL DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)	(time)	(date)	

PHYTOPLANKTON 3x1 SAMPLE LOG: CONTROL DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)	(time)	(date)	

Notes: _____

SOP 17 Operation of Ballast Water Sampling System (SHIPBOARD)

Application	LAND-BASED		SHIPBOARD	XX
-------------	------------	--	-----------	----

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Test Cycle Piping Diagrams identify system process points, instrumentation locations, and designate valve names. They are available in the automation system, on Facility laminated placards, and in key documents.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

Ballast Water Sampling System Operations

These procedures are performed to provide samples to support the analyses described in the Scientific Assays. The below table, Sampling Procedure Coordination, matches the pumping system operation to a particular sampling procedure and the resulting sample replicates and volumes.

Sampling Procedure Coordination Table

Pumping Operations		Sampling Procedures					
	Rate** (m3/hr)	Name	Location	Pitot/ Size (inches)	Each Replicate		
					Rate** (lpm)	Volume	
						10 - 50 µm	≥50 µm
Uptake							
Treatment (Untreated)	250	3 x 1	A1, A2, A3	S1 (1 x 1.61")	25	3 x 22 liters	3 x 1m3
Control (Untreated)	250	3 x 1	B1, B2, B3	S1 (1 x 1.61")	25	3 x 22 liters	3 x 1m3
Discharge							
Treatment (Treated)	250	3 x 3	A1, A2, A3	S4 (3 x 1.68")	75	3 x 22 liters	3 x 3m3
Control (Untreated)	250	3 x 1	B1, B2, B3	S1 (1 x 1.61")	25	3 x 22 liters	3 x 1m3

**During tank stripping pumping rates slows down.

Sampling Overview

Ballast water sampling procedures vary as per the ballasting operations as follows:

Ballast Water Uptake Untreated Water Samples (3 x 1m³ and 3 x 22 liters).

During ballast water uptake, untreated water is taken from the seachest and moved to the Treatment Tank, and then the Control Tank. During each of these sequential uptakes, three continuous samples of at least 1.0 cubic meter are taken before the BWTS. This method assures sampling during the entirety of both uptake events, and produces two sets of three (3) triplicate samples totaling not less than 3.0 cubic meters for analysis of the $\geq 50 \mu\text{m}$ size class, for each uptake.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from the same sample line. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 μm size class.

Ballast Water Discharge Treatment Tank Samples (3 x 3m³ and 3 x 22 liters).

During ballast water discharge from the Treatment Tank, integrated samples of treated water in triplicate of at least 3.0 cubic meters are each taken after the BWTS. This provides a total of three (3) 3.0 cubic meter samples for a total volume of at least 9.0 cubic meters. This method assures sampling during the entire discharge event for analysis of the $\geq 50 \mu\text{m}$ size class.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from the same sample line. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 μm size class.

Ballast Water Discharge Control Samples (3 x 1m³ and 3 x 22 liters).

During ballast water discharge from Control Tank, integrated samples of treated water are taken in triplicate of at least 1.0 cubic meters each. This provides a total of three (3) 1.0 cubic meter samples for a total volume of at least 3.0 cubic meters. This method assures sampling during the entire discharge event for analysis of the $\geq 50 \mu\text{m}$ size class.

At the same time triplicate carboys of 22 liters each are filled, unfiltered, from the same sample line. This produces three (3) triplicate samples of 22 liters each for analysis of the 10 – 50 μm size class.

Sampling Coordination

Ballast water uptake consists of one event that uptakes to the Treatment Tank (3-154-1) and then immediately uptakes to the Control Tank (3-154-2).

The BWTS inlet piping is primed, and water is pumped through a recirculation loop while the BWTS warms up. When the BWTS is ready, water is pumped to overboard (sea-to-sea). The sea-to-sea mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge from overboard to fill the Treatment Tank.

When the Treatment Tank reaches its designated volume, the team switches to start fill of the Control Tank while shutting down the BWTS. When the Control Tank reaches its designated volume, pumping is stopped to clean the piping system of control ballast water.

Ballast water discharge proceeds after ballast water is held for not-less-than 48 hours. Discharge includes two events that discharge the Treatment Tank, followed by the Control Tank.

The first event primes the BWTS inlet piping, starts the BWTS, and takes suction from the Treatment Tank. The treated water passes the BWTS, and returns to the treatment pump suction (recirculation). The recirculation mode enables the sampling team to set-up the sampling system. The pumping team then switches discharge to overboard, emptying the Treatment Tank to sea, while the sampling team takes triplicate sampling of the treated water. The Control Tank is recirculated, now by-passing the BWTS. The untreated control water is then sampled and discharged to sea.

Communications between the pumping team (opening and closing valves, operating the treatment system pump, and operating the BWTS) and the sampling team is performed by radio, visual communications, and the automation system ballast order telegraph (BOT). Of these, the BOT is the primary and definitive means of communication.

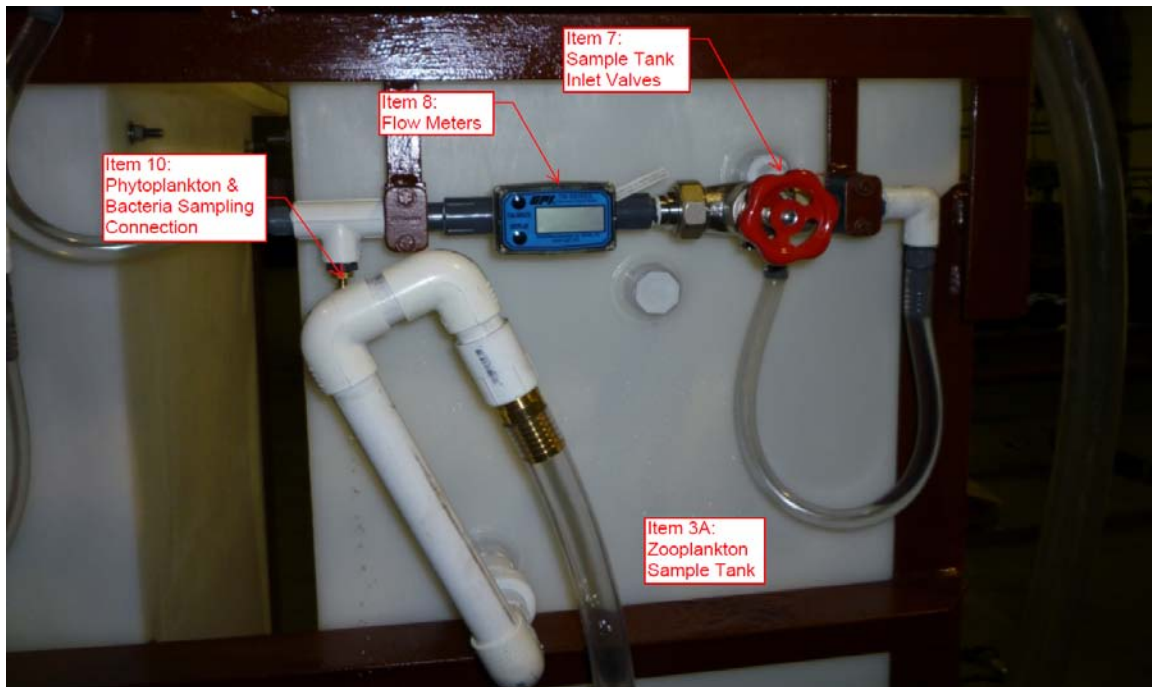
The sampling team will prepare the sampling system by flushing all equipment, hoses, and other devices with raw seawater before uptake or held ballasted before discharge. During set-up, the sampling system will also balance flows into the tubs where the plankton nets are rigged. However, the sampling team will only send ballast water through the plankton nets when directed by the pumping team. This is called “netting.” Only during an actual uptake or discharge are the samples netted, in order to maximize the sample representativeness of the ballast tank contents.

Uptake Set-up**Procedure 3 x 1 Set-up**

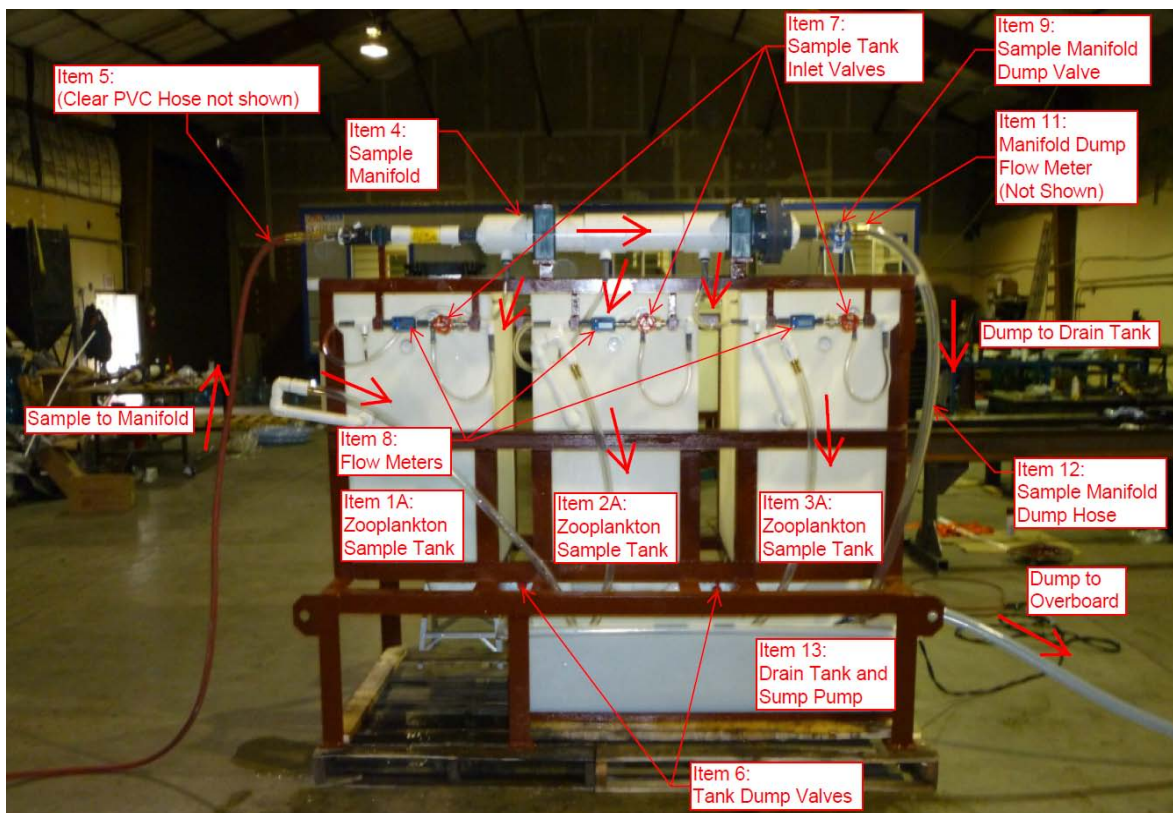
This step specifies critical equipment, pipe/hose connections, and system line-up required for 3 x 1m³ sampling.

- Ensure the manifold inlet piping is flushed with chlorinated fresh water and drained. The Dump Valve to the drain tank (Item 9) should be closed.
- Use the sample port located on the Main Deck (P&ID Position S1), with the 1-1/2" sampling pitot installed.
- Use sample assembly tubs as designated above for each process. Align (but do not connect) sample port S1 to the Sample Manifold (Item 4) using 1-1/2" Clear PVC Hose (Item 5).
- Prepare the six (6) Zooplankton Sample Tubs (Items 1, 2, & 3).
 - Each tub should be rinsed with fresh water and drained.
 - Inspect internal and external tubing and piping to ensure connection to the sample manifold and drain tank.
 - The Sample Tub Inlet Valves (Item 7) should be SHUT. The Tub Dump Valves (Item 6) should be SHUT, allowing the weir pipe to engage.
 - Install the collection nets in each of the three Zooplankton Sample Tubs.
- Ensure all Flow Meters (Items 8 & 11) are operational.
- Connect Drain Tank Sump Pump (Item 13) to power, and connect Sump Pump "Dump to Overboard" line to a waste connection.

Notes: _____



Sample Tank Inlet Pipe Construction (Typ.)



Sampling Assembly (Assembly "A" Shown)

SOP 17, Step 1 Uptake Sampling Flushing

Uptake sampling is taken by filling all three tubs during the sequential Treatment uptake, and then the Control uptake. The following prepares the tubs.

Sample Pitot Isokinetic Flow Rates – Uptake Mode

Flow Rates for 3.6m ³ Sample w/ 1.61 Inch I.D. Pitot at 48 Minutes				
Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate/ Sample to Manifold (gpm)	4.0	19.1	19.9	20.7
Throttle Flow Meters as per Table				
Tub 1 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 2 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 3 Rate (gpm)*	1.33	6.37	6.64	6.90

- Lead Operator will indicate on BOT “Uptake Sea-to-Sea.” This indicates that the sampling system can now be flushed and prepared.
- Flush sample port S1 hose over the side for not less than one minute, and until relatively clear water is achieved.
- Connect sample port S1 to Sample Tub Manifold A. Open the Sample Manifold Dump Valve (Item 9). Ensure the Sump Pump and Float Switch assembly (Item 13) is operational.
- Open the Sample Tank Inlet Valve (Item 7) for A1, A2, and A3 and adjust weir pipes to ensure the following:
 - Keep net bodies out of the influent flow. Water should flow into the tubs.
 - Collection net bodies should be “flooded” with the sample water in the tubs.
 - Collection net tops should be above the top of the tub water level.
 - Collection piping should be submerged about 2 inches from the net cod end, so that sample entry into the tubs is gentle.
 - Collection net cod end should be about 3 inches from the tub bottom.
- Prepare tubs A1, A2, and A3 for zooplankton collection.
 - Adjust flow rate into tubs (but not through net) according to the flow rate chart (above).
 - Reset flow meter totalizer.
- Using BOT report to Lead Operator “Ready to Sample.”

SOP 17, Step 2 Uptake Sampling

The BWTS will have already been placed online during the recirculation operation. Sampling begins immediately before uptake actually starts and finishes when both tanks are filled.

Zooplankton Sampling

- The sampling team, as per previous step, should be in a position of “Ready to Sample” and indicate this on the BOT. The pumping team will be indicating “Uptake Sea-to-Sea” on the BOT.
- The Lead Operator will indicate on BOT “Treatment Uptake” to signal for the sampling team to start “netting.”
- The sampling team will immediately start netting at this BOT signal, log the netting into the automation system, and use the BOT to match the appropriate pumping team signal, Treatment Uptake.
- If sample net becomes overwhelmed with material, close the Sample Tank Inlet Valve (Item 7), remove sample net, replace with a new net, open the Sample Tank Inlet Valve, and log event.
- When Lead Operator indicates that the Treatment Uptake event has been completed, stop netting in tubs A1, A2, A3, and start netting in tubs B1, B2, B3 as the pumping operation switches to Control Uptake. When the lead Operator indicates on BOT “Control Uptake”, match the appropriate signal.
- When Lead Operator indicates that Control Uptake event has been completed, stop netting, and log event.

Phytoplankton and Bacteria Sampling

- Use small tubing lines attached to the Phytoplankton & Bacteria Sampling Connection (Item 10) to gain samples for phytoplankton and bacteria.
- Gain one continuous sample from each tub for a total of three (3) continuous samples. Each of these samples should cover the entire ballasting event, either as a periodic fill such that eventually the full sample is obtained, or as a continuous drip feed.
- Log sampling times for each of the three (3) Phytoplankton samples. Label each collected sample.

SOP 17, Step 3 Uptake Stopping and Sample Handling

Stop and secure the ballast sampling system, and process the samples as indicated below.

Stopping Sampling

- On uptake, sampling shall be stopped at completion of the event by direction of the Lead Operator.
- Close the Sample Manifold Dump Valve (Item 9) and local valve at Pitot Tube (see Sample Assembly Construction figure, and allow the Sump Pump to empty the Drain Tank (Item 13) automatically.
- Disconnect the 1-1/2 inch PVC hose from the Sample Manifold (Item 4) to avoid contamination.
- Rinse plankton nets by gently submerging and lifting in full sample tub. Examine net using microscopy to confirm “loss percentage” of organisms.

Sample Processing

- Samples must be clearly labeled and immediately brought to the Biological Laboratory for processing.
- Sample handling and analysis protocols are provided in a separate document.

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

SOP 17, Step 4 Cleaning

After collecting the ballast samples, clean the system as soon as possible as indicated below to prevent fouling or organism growth in the system.

- Drain all tanks, hoses, manifolds;
- Immediately flush with mild fresh water and bleach solution;
- Drain all components;
- Discard any components which are fouled and not readily cleaned.

Notes: _____

Discharge Set-up**Procedure 3x3 Set-up (Prior to Discharge Operations)**

This step specifies critical equipment, pipe/hose connections, and system line-up required for 3x3 sampling.

- Ensure that tank inlet piping is flushed with chlorinated fresh water and drained. The Dump Valve to the drain tank (item 9) should be closed.
- Use the sample port located on the Main Deck (P&ID Position S4), with the triple 1-1/2 sampling pitot installed.
- Use sample assembly tubs as designated above for each process. Align (but do not connect) each pipe of sample port S4 to the inlet piping of each sample tub, using 1 inch clear PVC hose. The hose should be connected directly to the sample tub inlets, in way of the sampling manifold.
- Prepare the three (3) Zooplankton Sample Tubs (Items 1, 2, & 3).
 - Each tub should be rinsed with fresh water and drained.
 - Inspect internal and external tubing and piping to ensure connection to the sample tubing and drain tank.
 - The Sample Tub Inlet Valves (Item 7) should be SHUT. The Tub Dump Valves (Item 6) should be SHUT, allowing the weir pipe to engage.
 - Install the collection nets in each of the three Zooplankton Sample Tubs.
- Ensure all Flow Meters (Item 8) are operational.
- Connect Drain Tank Sump Pump (Item 13) to power, and connect Sump Pump “Dump to Overboard” line to a waste connection.

Procedure 3x1 Set-up (Prior to Discharge Operations)

This step specifies critical equipment, pipe-hose connections, and system line-up required for 3x1 sampling.

- Ensure the manifold inlet piping is flushed with chlorinated fresh water and drained. The Dump Valve to the drain tank (Item 9) should be closed.
- Use the sample port located on the Main Deck (P&ID Position S1), with the 1-1/2 sampling pitot installed.
- Use sample assembly tubs as designated above for each process. Align (but do not connect) sample port S1 to the Sample Manifold (Item 4) using 1-1/2 inch Clear PVC Hose (Item 5).
- Prepare the three (3) Zooplankton Sample Tubs (Items 1, 2, & 3).
 - Each tub should be rinsed with fresh water and drained.
 - Inspect internal and external tubing and piping to ensure connection to the sample manifold and drain tank.

- The Sample Tub Inlet Valves (Item 7) should be SHUT. The Tub Dump Valves (Item 6) should be SHUT, allowing the weir pipe to engage.
- Install the collection nets in each of the three Zooplankton Sample Tubs.
- Ensure all Flow Meters (Items 8 & 11) are operational.
- Connect Drain Tank Sump Pump (Item 13) to power, and connect Sump Pump “Dump to Overboard” line to a waste connection.

Notes: _____

SOP 17, Step 5 Discharge Flushing

Treatment Discharge Flushing

Treatment Discharge sampling is taken by filling THREE tubs for Treatment Discharge, of 3.0 cubic meters each. The Treatment Discharge is sampled with the 3x3 procedure.

Sample Pitot Isokinetic Flow Rates – Treatment Discharge Mode

Flow Rates for 10.8m³ Sample w/ 3x1.68 Inch I.D. Pitots at 48 Minutes				
Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate per Pitot/ Sample to Tanks (gpm)	4.0	19.0	19.8	20.6
Throttle Flow Meters as per Table				
Tub 1 Rate (gpm)*	3.97	19.04	19.83	20.63
Tub 2 Rate (gpm)*	3.97	19.04	19.83	20.63
Tub 3 Rate (gpm)*	3.97	19.04	19.83	20.63

- Lead Operator will indicate on BOT “Treatment Recirculation.” This indicates that the sampling system can now be flushed and prepared.
- Flush sample port S4 hoses over the side for not less than one minute, and until relatively clear water is achieved.
- Connect sample port S4 hoses to the Sample Tank A inlets. Ensure the Sump Pump and Float Switch assembly (Item 13) is operational.
- Open each Sample Tub Inlet Valve (Item 7) for A1, A2, and A3, throttling to achieve tub flow rates to each sample tub as specified by the flow rate chart (above).
- Disconnect sample port hoses from the sampling tubs, and alert Lead Operator via radio to switch to Discharge to flush system.
 - System will be discharged to overboard for not less than one minute, and until relatively clear water is achieved.
 - Once flushing is completed, reconnect sample port hoses and alert lead Operator to Switch back to recirculation.
- Shut the Tub Dump Valves (Item 6) and adjust weir pipes to fill the tubs, and ensure the following:
 - Keep net bodies out of the influent flow. Water should flow into the tubs.
 - Collection net bodies should be “flooded” with the sample water in the tubs.
 - Collection net tops should be above the top of the tub water level.
 - Collection piping should be submerged about 2 inches from the net cod end, so that sample entry into the tubs is gentle.

- Collection net cod end should be about 3 inches from the tub bottom.
- Prepare tubs A1, A2, and A3 for zooplankton collection.
 - Adjust flow rates into each tub (but not through net) according to the flow rate chart (above).
 - Reset flow meter totalizer.
 - Monitor and balance flow in each tub as necessary.
- Using BOT report to Lead Operator “Ready to Sample.”

Control Discharge Flushing

Control Discharge sampling is taken by filling THREE tubs of 1 cubic meter each. The Control Discharge is sampled with the 3x1 procedure.

Sample Pitot Isokinetic Flow Rates – Control Discharge Mode

Flow Rates for 3.6m3 Sample w/ 1.61 Inch I.D. Pitot at 48 Minutes				
Total Ballast Flow Rate (cu m/hr)	50	240	250	260
Total Ballast Flow Rate (gpm)	220	1057	1101	1145
Isokinetic Flow Rate/ Sample to Manifold (gpm)	4.0	19.1	19.9	20.7
Throttle Flow Meters as per Table				
Tub 1 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 2 Rate (gpm)*	1.33	6.37	6.64	6.90
Tub 3 Rate (gpm)*	1.33	6.37	6.64	6.90

- Lead Operator will indicate on BOT “Control Recirculation.” This indicates that the sampling system can now be flushed and prepared.
- Flush sample port S1 hose over the side for not less than one minute, and until relatively clear water is achieved.
- Connect sample port S1 to Sample Tub Manifold B. Ensure the Sump Pump and Float Switch assembly (Item 13) is operational.
- Open each Sample Tub Inlet Valve (Item 7) for B1, B2, and B3, throttling to achieve tub flow rates to each sample tub as specified by the flow rate chart (above).
- Disconnect sample port hose from manifold, and alert Lead Operator via radio to switch to Discharge to flush system.
 - System will be discharged to overboard for not less than one minute, and until relatively clear water is achieved.
 - Once flushing is completed, reconnect sample port hose and alert Lead Operator to switch back to recirculation.
- Shut the Tub Dump Valves (Item 6) and adjust weir pipes to fill the tubs, and ensure the following:

- Keep net bodies out of the influent flow. Water should flow into the tubs.
 - Collection net bodies should be “flooded” with the sample water in the tubs.
 - Collection net tops should be above the top of the tub water level.
 - Collection piping should be submerged about 2 inches from the net cod end, so that sample entry into the tubs is gentle.
 - Collection net cod end should be about 3 inches from the tub bottom.
- Prepare tubs B1, B2, and B3 for zooplankton collection.
 - Adjust flow rates into each tub (but not through net) according to the flow rate chart (above).
 - Reset flow meter totalizer.
 - Monitor and balance flow in each tub as necessary.
- Using BOT report to Lead Operator “Ready to Sample.”

SOP 17, Step 6 Discharge Sampling

Sampling shall begin immediately after the treatment system is started and operating at steady state, and just before the ballast treatment recirculation mode has been secured, and test cycle is started. The sampling procedure shall be repeated sequentially for Treatment Discharge and Control Discharge.

Zooplankton Sampling

- The sampling team, as per previous step, should be in a position of “Ready to Sample” and indicate this on the BOT. The pumping team will be indicating “Recirculation” on the BOT.
- The Lead Operator will signal via the BOT when to start “netting” as follows:
 - Treatment Discharge – Use Tubs A1, A2, and A3
 - Control Discharge – Use Tubs B1, B2, and B3
- The sampling team will immediately start netting at these BOT signals, log the netting into the automation system, and use the BOT to match the appropriate pumping team signal, i.e. Treatment Discharge.
- If a sample net becomes overwhelmed with material, close the Sample Tank Inlet Valve (Item 7), remove sample net, replace with a new net, open the Sample Tank Inlet Valve, and log event.
- **Note:** At end of the each discharge cycle (Treatment, Control), the system flow will be reduced and isokinetic sample flow will be decreased as follows.
 - With approximately 1 foot remaining in tank, Lead Operator will reduce flow in system to 50 m³/hr while maintaining 200-250 kPa at position 2. There will remain 10-15 minutes estimated discharge time and sampling will be adjusted accordingly.
 - Adjust the Sample Tub Inlet Valves (Item 7) to each of the three (3) Zooplankton Sample Tanks (Items 1, 2, & 3), throttling to maintain tank flow rates to each sample tub as specified by the Tub Rates in the Sample Pitot Isokinetic Flow Rates tables.
- When Lead Operator indicates that a discharge event has been completed, stop netting, and log event.
- Repeat the process for each of the two discharge events.

Phytoplankton and Bacteria Sampling

- Use small tubing lines attached to the Phytoplankton & Bacteria Sampling Connection (Item 10) to gain samples for phytoplankton and bacteria.
- Gain three (3) continuous samples for each discharge event, one from each sampling tub. Each of these samples should cover the entire ballasting event, either as a periodic fill such that eventually the full sample is obtained, or as a continuous drip feed.
- Log sampling times for each event’s three (3) Phytoplankton samples. Label each collected sample.

Test Cycle Number

PIC Initials

Quality Initials

Date

Notes: _____

Stopping and Sample Handling

Stop and secure the ballast sampling system, and process the samples as indicated below.

Stopping Sampling

- On discharge events, with 1 foot remaining in tank flow will be reduced to 50 m³/hr and sample flow will be reduced proportionally. When flow is lost approximately 10-15 minutes after this flow reduction, the sample inlet valves are immediately shut.
- Close the local valves at Pitot Tube, and allow the Sump Pump to empty the Drain Tank (Item 13) automatically.
- Disconnect the 1 inch PVC hoses from the Sample Tank A inlets and the 1-1/2 inch PVC hose from Sample Manifold B to avoid contamination.
- Rinse plankton nets by gently submerging and lifting in full sample tub. If required (from visual inspection), perform a final rinse down to cod-end by use of a hand pump sprayer filled with sample tub water.

Sample Processing

- Samples must be clearly labeled and immediately brought to the Biological Laboratory for processing.
- Sample handling and analysis protocols are provided in a separate document.

Notes: _____

Cleaning

After collecting the ballast samples, clean the system as soon as possible as indicated below to prevent fouling or organism growth in the system.

- Drain all tanks, hoses, pipes;
- Immediately flush with mild fresh water and bleach solution;
- Drain all components;
- Discard any components which are fouled and not readily cleaned.

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Uptake Sample Collection Logs

ZOOPLANKTON 3x1 SAMPLE LOG: UPTAKE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial) (time) (date)			

PHYTOPLANKTON 3x1 SAMPLE LOG: UPTAKE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial) (time) (date)			

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Treatment Discharge Sample Collection Logs

ZOOPLANKTON 3x3 SAMPLE LOG: TREATMENT DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)		(time)	(date)

PHYTOPLANKTON 3x3 SAMPLE LOG: TREATMENT DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)		(time)	(date)

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Control Discharge Sample Collection Logs

ZOOPLANKTON 3x1 SAMPLE LOG: CONTROL DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)	(time)	(date)	

PHYTOPLANKTON 3x1 SAMPLE LOG: CONTROL DISCHARGE			
Tanks / Label Name			
Start Time			
Flow Rate (gpm)			
End Time			
Total Volume (gal.)			
(initial)	(time)	(date)	

Notes: _____

SOP 18 Chain of Custody

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

[illegible]

SOP 19 Biological Analysis Data Sheets				
Application	LAND-BASED	XX	SHIPBOARD	XX

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as “Support.” Each sheet that they entered data shall also bear their name, noting “data entry by” and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and Online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write “ONLINE RECORD” on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____

Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date

Overview

The following checklists and logs are provided to track biological efficacy analysis.

Poke and Probe Viability Determination for Organisms $\geq 50 \mu\text{m}$ (SOP 15)

Counter:								
Date:								
Location/Replicate	BW Filtered (L)	Cod End Volume (mL)	FSW Added (mL)	SW Analyzed (mL)	Alive	Dead	Actual Concentration	Misc. Notes

BW=Ballast water, FSW=Filtered seawater, SW=Seawater

Notes: _____

Test Cycle Number

PIC Initials

Quality Initials

Date _____

Chlorophyll a Checklist (SOP 18)

Check before run:

Air On

Flow Rates

Purged*

Solvent Levels

Solvent Waste

AS3000 Waste

Pressure % A

Pressure % B

Things to double

check before run:

1. Sample, empty, etc. in AS3000 + 1 ACTN in last spot?

2. 350 μ in vials?

Things to

remember:

- AS3000 = Pull Loop

- Normal psi:

Solvent A = 2.900

Solvent B = 1,150

-Solvent 1 = DI H₂O

-Flush Bottle = MeOH

*** Solvent A and B
need to be manually
purged if:**

1. Solvent has been run dry or fret

exposed to air

2. HPLC has been shut down completely (solvents no longer degassed)

Gradient other than

Zapata note here:

Sequence Queue	Sample Description
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	
11.	
12.	
13.	
14.	
15.	
16.	
17.	
18.	
19.	
20.	
21.	
22.	
23.	
24.	
25.	
26.	
27.	
28.	
29.	
30.	
31.	
32.	
33.	
34.	
35.	
36.	
37.	
38.	
39.	
40.	
41.	
42.	
43.	
44.	
45.	
46.	
47.	
48.	
49.	
50.	

Notes	

Test Cycle Number

PIC Initials

Quality Initials

Date

FDA-Based, Flow Cytometric Analysis of Viable Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$ (SOP 22)

Date:	
File Folder:	
File Prefix:	

User:

Instrument Settings:		
	Lon/lin	threshold
FSC=		
SSC=		
FL1=		
FL2=		
FL3=		

Instrument Settings:		
	Lon/lin	threshold
FSC=		
SSC=		
FL1=		
FL2=		
FL3=		

Instrument Settings:		
	Lon/lin	threshold
FSC=		
SSC=		
FL1=		
FL2=		
FL3=		

File	Sample	Settings
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		

File	Sample	Settings
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		

File	Sample	Settings
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		

Flow Rate Calibration		
Time of Calibration:		
Wi=	Wf=	
T =		
Rate =		
Time of Calibration:		
Wi=	Wf=	
T =		
Rate =		

Flow Rate Calibration		
Time of Calibration:		
Wi=	Wf=	
T =		
Rate =		
Time of Calibration:		
Wi=	Wf=	
T =		
Rate =		

Flow Rate Calibration		
Time of Calibration:		
Wi=	Wf=	
T =		
Rate =		
Time of Calibration:		
Wi=	Wf=	
T =		
Rate =		

SOP 20 Poke and Probe Viability Determination for Organisms $\geq 50 \mu\text{m}$

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Organisms will be inspected under moderate power dissecting microscopes (nominally 30x) to determine concentrations of live and dead organisms $\geq 50 \mu\text{m}$ in minimum dimension. The samples will be collected in zooplankton nets constructed of mesh with an open square aperture $35 \mu\text{m}$ on each side ($50 \mu\text{m}$ on diagonal).

Method References

EPA/ETV Generic Protocol for the Verification of Ballast Water Treatment Technologies, v4.2 (2010).

Lee II, H., D.A. Reusser, M. Frazier, and G.M. Ruiz. 2010. Density matters: Review of approaches to setting organism-based ballast water discharge standards. U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Western Ecology Division. EPA/600/R-10/31.

Sampling Procedures

Samples for organisms $\geq 50 \mu\text{m}$ in minimum dimension will be collected in custom constructed zooplankton nets, designed to fit within the tub size of the GBF biological sample tanks. The nets are built by Research Nets, Inc., Seattle, WA to our specifications. The mesh size will be $35 \mu\text{m}$ Nitex material, with all seams double stitched. Mouth size of the net is 40 cm, with right-cylinder mesh construction running $\frac{3}{4}$ of the length of the net to maximize filtration area. The removable cod-end will be fit with the same $35 \mu\text{m}$ mesh on one side of the cod-end to facilitate sample drainage and transfer to holding jars.

The description of sample collection is fully elaborated elsewhere. Briefly, we will collect continuous samples over the full 2 hour ballasting period in each of three replicate nets. The flow rates (in isokinetic sampling mode) will be metered to yield, at minimum, 1 m^3 in each net, for both the uptake operation and for the discharge of the control tank. During discharge of the treatment tank at least 3 m^3 will be collected in each net (again, three replicates).

The zooplankton nets will be rinsed with sample water in the full tubs at the end of each ballasting operation to ensure all organisms are collected in the cod-end. The net contents will be transferred to clean, wide-mouth glass jars to a final volume of 400 mL (measured volumetrically). The zooplankton samples will be kept at refrigerator temperature during the counting procedure.

Analytical Procedures

Two zooplankton counting technicians will inspect the samples simultaneously at independent microscope stations. Live counts in the uptake sample and the control tank sample are expected to be high ($>100,000 \text{ m}^{-3}$). Sample jars will be stirred and subsampled

with 5 mL pipets to fill each of two, 10 mL Bogorov serpentine counting chambers. If the samples are too concentrated to count, quantitative dilutions will be made with filtered (0.22 μm) sample water such that at least 100 live animals are present in each 10 mL counting tray. Each analyst will count three, 10 mL subsamples, scoring the live and dead status of all organisms $\geq 50 \mu\text{m}$ in minimum dimension. Live organisms will be identified by swimming motion, internal organ movement, or escape upon probing.

Samples from the treatment ballast tank are expected to have low total zooplankton counts (due to treatment filtration) and low live counts (due to treatment biocidal effects). Therefore, in order to increase the observed count rates, each 400 mL net sample from the treatment tank will be reduced in volume through 35 μm sieves to yield 60 mL final volume. This will provide each analyst with three, 10 mL aliquots and will result in complete inspection of the entire net sampling volume ($3 \times 3 = 9 \text{ m}^3$). Live counts for the treatment ballast tanks will be pooled for all replicates and treated in Poisson statistical analysis for rare counts as described in Lee et al. (2010).

Following organism enumeration, two separate samples will be preserved in 4% buffered formalin and stored for at least six (6) months.

SOP 21 Science Assay — Most Probable Number (MPN) Determination of Viable Phytoplankton Cells ≥ 10 to $< 50 \mu\text{m}$, Chlorophyll-based

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Viable organisms ≥ 10 but $< 50 \mu\text{m}$ (hereafter referred to as ≥ 10 to $< 50 \mu\text{m}$) are difficult to enumerate quantitatively because live and dead cells in this size class cannot be distinguished visually. Therefore, we have assembled several independent methods, based on culture methods and/or viability stains, that attempt to enumerate viable organisms in the ≥ 10 to $< 50 \mu\text{m}$ size class to meet the demands of ballast regulations that specifically call for numeric ‘live’ counts.

Here we describe a culture-based, most probable (MPN) technique to enumerate photoautotrophic organisms in the size range ≥ 10 to $< 50 \mu\text{m}$. This method is conservative since it evaluates only those organisms that can grow photoautotrophically; colorless heterotrophs in this size class are not considered.

In this method we concentrate organisms onto $10 \mu\text{m}$ Nitex filters, resuspend the cells into a concentrated fluid stock, and serially dilute the stock sample into an MPN culture tube matrix with 6 replicates at each of 7 dilutions plus one replicated series of ‘blank’ media. The tubes are incubated under continuous illumination and measured fluorometrically for evidence of cell growth, defined by chlorophyll increases, over a period of 14 days.

Method References

Woomer, P., J. Bennett, and R. Yost. 1990. Overcoming the inflexibility of most-probable-number procedures. *Agron. J.* 82: 349-353.

Sampling Procedures

The MPN tube array utilized here is a 5x8 matrix of clear growth tubes, each holding 3 mL sample fluid. There are 5 replicate tubes for each of 6 serial dilutions; the 1st row consists of raw sample water, the 8th row consists of growth media only, to serve as a blank. Each row in the MPN matrix represents a 5-fold dilution of the previous row. Thus, the dilution series represents a 4-log reduction ($5^6=15625$) of the original sample water which provides adequate resolution to determine both challenge water viable concentrations (expected to be >100 viable organisms mL^{-3}) and treatment concentrations (expected to be $<<10 \text{ mL}^{-3}$). A 40-tube (5x8) MPN matrix will be prepared for each of three replicate uptake samples, three replicate control tank samples and three replicate treatment tank samples.

Prepare sterilized, nutrient enriched growth media for all MPN experiments using one source of original natural water, specifically, the uptake water. (The MPN culturing technique relies on measurable cell growth as the analytical ‘score’ for viability; therefore, treatment water must NOT be used to make growth media. Likewise, we will avoid using control water that had been held in the control ballast tank since it might have been in contact with metals and antifouling paint on the interior of the tank). A total of 3 L growth

media (allowing ample extra) will be needed to make up MPN arrays for all three phases of the ballasting operation, e.g., uptake, control, and treatment. Filter uptake water through a flow-through 0.22 μm filter using a Masterflex peristaltic pump, fit with clean silicone tubing. Add concentrated growth media to yield a final F/8 concentration of Guillard's enriched seawater media (Sigma). Divide enriched media into three, 1 L batches in glass vessels; microwave each 1 L batch under full power for two minutes. Let the media cool for 30 min, repeat three more times so that each media batch has been microwave sterilized for 4 consecutive 2 min. periods (Keller 1980).

Using sterile volumetric pipets, dispense 2.4 mL sterile media into all replicate tubes in rows 2-7; pipet 3 mL into row 8 (the 'blank'). We recommend including a 6th replicate tube in each row that will serve as a convenient sample source for each subsequent row when the dilutions are being prepared. Tubes are now ready for sample additions and dilutions.

Approximately 100 mL of sample water will be needed for each MPN array; measure sample volumetrically and record in databook. The organisms from at least 100 mL sample water will be concentrated onto custom-cut 10 μm Nitex filters by gravity filtration. The filters will be diluted back to 100 mL with sterile media in a suitable vessel (acid cleaned, graduated 125 mL polycarbonate bottle) and vortexed to resuspend particles from the filter, thus isolating the organisms $\geq 10 \mu\text{m}$ back to their original concentration. The purpose of this filtration step is to remove organisms $\geq 10 \mu\text{m}$ from the assay.

Pipet 3 mL of the resuspended sample into a 6 replicates in row 1. Cap tubes with snap caps. Use the 6th replicate of row 1 as a sample source for row 2; add 0.6 mL to each media tube in Row 2. Cap and invert tube to mix. This yields a 5-fold dilution in each tube of row 2, with a final 3 mL volume. Repeat through row 7. Cap all tubes including the blank media in row 8.

Analytical Procedure

Measure the fluorescence of each tube in the Spex Fluorolog spectrofluorometer (430 nm excitation/680 nm emission; 5 nm bandwidth. Each clear culture tube serves as its own fluorometer cuvet). Use the constant-wavelength analysis software module to automatically record all readings in convenient spreadsheet form. This sets the 'time-zero' fluorometric chlorophyll readings for all tubes (including blanks) in the MPN array. Place MPN arrays in illuminated, temperature controlled incubator set to ambient seawater temperature. Record fluorescent reading again on Day 3 and Day 5. Positive growth is indicated by at least a two-fold increase in fluorescence relative to the 'time-zero' measurement for each tube.

Following organism analysis, two separate samples will be preserved in 1% glutaraldehyde and stored for at least six (6) months.

Equipment Notes

Spex Fluorolog 2 spectrofluorometer is calibrated for excitation wavelength and emission wavelengths using lamp scan and Raman water scans as per instruction posted on front of instrument. Raman fluorescence response at 350nm/397nm (ex/em) determines instruments response sensitivity; record signal in field notebook at the time of analysis.

SOP 22 C-14 Primary Production

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

The radiotracer C-14 technique will be applied to uptake, control and treatment samples (each in triplicate) to yield physiological measurements of photosynthesis (carbon fixation rates). Experiments will be initiated and terminated at MLML in a walk in cold room nominally held at 13 °C. Samples will be prepared in triplicate, acid-washed polycarbonate bottles (125 mL), inoculated with C-14 (2 µCi) and incubated for 24 hours under continuous, constant illumination provided by high intensity LED lamps; bottles will be rotated continuously on motorized plankton wheels to ensure uniform irradiance exposure and to prevent settling of cells. C-14 processing will follow that of Welschmeyer et al. (1993). Whole water sample aliquots will be harvested onto GF/F filters (0.7 µm) and 10 µm nylon filters to estimate total and >10 µm photosynthetic rates, respectively (µgC L⁻¹ d⁻¹). Total dissolved inorganic carbonate (DIC) will be determined on a UIC CM5012 CO₂ Coulometer for proper determination of DIC specific activity (dpm/gC). Chlorophyll specific photosynthetic rates will be computed from Chl measurements made on the same water samples.

Following organism analysis, two separate samples will be preserved in 1% glutaraldehyde and stored for at least six (6) months.

Method References

Welschmeyer, N.A., S. Strom, R. Goericke, G. DiTullio, M. Belvin and W. Peterson. 1993. Primary production in the subarctic Pacific Ocean: Project SUPER. Progress in Oceanography 32: 101-136.

SOP 23 Science Assay — Chlorophyll *a*

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Chlorophyll *a*, determined from solvent extraction fluorometric analysis, is used as a bulk measure of relative phytoplankton concentrations in uptake, control and treatment ballast water.

Method References

Welschmeyer (1994)

Sampling Procedures

- Filter water samples volumetrically under vacuum not to exceed one-third atmosphere (e.g., 25 cm Hg; 10 in Hg; 5 psi); triplicate samples should be processed; nominally, a 135mL filtration of SF Bay water should be adequate. Keep filtration times for each sample <15min by adjusting volumes accordingly; record volumes for each sample.
- Immerse filter into screw-cap microcentrifuge tubes, each pre-filled with 1.2 mL 90% acetone; make certain the filter is covered fully by extraction fluid. Immediately store extraction tubes in -20 °C freezer until analysis time. Fluorometric chlorophyll samples will likely be run on board ship to provide preliminary assessment of ballast treatment performance. Fluorometric chl *a* can be determined after an overnight extraction period. Vortex each extraction tube for 5 seconds under full vortex speed. Pack the filter into bottom of microcentrifuge extraction tube with stainless steel spatula; invert tube gently to mix fluid contents. Centrifuge at 10,000 RPM for 2 min. to remove particles. Extracts are now ready for fluorometric analysis.
Important: aliquots for both a) fluorometric and b) HPLC pigment analyses will be derived from the same extraction tube; record all volumes removed so that subsequent introduction of HPLC internal standards can be properly quantified.
- Following organism analysis, two separate samples will be preserved in 1% glutaraldehyde and stored for at least six (6) months.

Analytical Procedures (fluorometry)

Remove 100 uL (nominally) of extract and dispense into fluorometer cuvetts filled volumetrically with 90% acetone (nominally, 5 mL). Shake tube, read on calibrated Turner TD-700 fluorometer fit with single-step optical filters as per Welschmeyer (1994). Record volumes removed from extraction tubes and dilution factors (5100/100) on sample data sheets. Read fluorescence blanks from three replicate cuvetts filled with 5 mL 90% acetone; read fluorescence from permanent secondary standard on high and low optical setting. Chl *a* is calculated directly from the empirical response factor (RF) posted on the instrument; permanent secondary standards provide quantitative monitoring of potential instrument drift. Chl *a* standards will be quantified spectrophotometrically at 664nm using a specific absorption coefficient of 87.67 L g⁻¹ cm⁻¹. Chl *a* standards in 90% acetone will be dated upon preparation and maintained at -20C at all times.

Equipment Notes

Turner TD700 Fluorometer. The fluorometer for chl a readings will be calibrated for chlorophyll a response factor no earlier than 3 months prior to commencement of the ballast test series. The instrument response factor [(ug Chl a/mL)/raw fluorescence] will be posted on the fluorometer case along with the corresponding fluorescence value of the permanent fluorescence standard. On the day of analyses, the permanent fluorescent standard will be read and recorded in field notebook; the instrument is performing adequately if the reading is within 15% of the posted calibration data.

Thermo Separation Products HPLC. Instrument is maintained by MLML staff.

Centrifuge. Non-critical instrument operated at 3000 RPM to remove filter pulp from optical path.

Pipets. 200 uL Gilson pipet will be gravimetrically calibrated and posted with verification date no earlier than 3 months prior to ballast experiments.

Chl a standard. Stock chl a (Sigma) will be diluted appropriately with 90% acetone, checked for chromatographic purity on HPLC and calibrated from measured optical density at 664nm, specific absorption coefficient= $87.67 \text{ L g}^{-1} \text{ cm}^{-1}$.

**SOP 24 Heterotrophic Bacteria Plate Counts for Organisms
<10 µm**

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Agar based plate counts of cultivable bacteria provide a simple means of evaluating ballast treatment efficacy. It is well recognized that the colony forming units (CFU) generated in plate counts seriously underestimate total bacterial densities by several orders of magnitude. However, the results still provide vivid visual indication of treatment success and with reasonable replication and digital colony counting precision a useful quantitative disinfection efficiency for culturable bacterial can be computed. The simple streak plate culture experiment is described below.

Method References

EPA/ETV Generic Protocol for the Verification of Ballast Water Treatment Technologies, v4.2 (2010).

Sampling Procedures

Prepare Difco Marine Agar 2216 according to instructions on the package label. When autoclaved agar has cooled enough to handle safely, pour agar into sterile plastic 100mm petri plates, cover and let agar solidify at room temperature. Stack plates and hold at room temperature for several days, checking for possible colony contamination. Select all clean petri plates and store in refrigerator until needed.

Acid wash bacterial sample bottles (1 L) before each ballasting operation.

Set up alcohol lamp and clean working surface in a draft free room. Use hockey stick glass rod and petri plate spin disk to spread 100 µL ballast water over complete center surface of agar. Flame glass rod in 95% isopropyl alcohol before each spread preparation. Produce at least three replicate plates for each ballast sample. Label each petri dish on the bottom outer edge of the lower agar plate.

Place petri plates in darkened, room temperature cabinet for 24 h.

Analytical Procedure

Determine colony counts after 24 h from digital images taken with the Bio Rad Fluor S-Max Image Analyzer. Adjust camera focus and contrast to obtain the sharpest colony images possible, setting the plates upside down so that the sample name is legible and recorded in digital image. Once set, scan all plates under the same conditions.

Adjust colony count software to recognize colonies with minimum detection of 'noise spots'. Strive to analyze all plates under the same colony detection sensitivity settings if possible.

SOP 25 Indicator Microbes *E. coli* and Enterococci for Organisms <10 µm

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Water quality indicator microbes *Escherichia coli* and *Enterococci* are to be enumerated using commercially available MPN kits from Idexx, Inc. The Colilert® kit provides MPN estimates of total coliforms and *E. coli*; Enterolert® kit provides MPN estimates of *Enterococci*. Both kits are based on species-specific chromogenic reactions that can be visualized with the naked eye and/or handheld blacklight illumination.

Method References

EPA/ETV Generic Protocol for the Verification of Ballast Water Treatment Technologies, v4.2 (2010).

Sampling Procedures

Acid wash collection carboys before each ballasting operation. (Hot water scrub, 10% HCl rinse, ten Nanopure rinses; cap the carboys and cover spigot with polyethylene glove to prevent contamination). Subsample three replicate 100 mL volumes into sterilized plastic sample bottle (Idexx) for each microbe container collection; 9 replicates for uptake, 9 for control, 9 for treatment. Prepare Colilert and Enterolert Quanti-Trays as per instructions supplied with each Idexx packaging box. Make sure the heated Quanti-Tray sealer has free bench space for insertion and exit of trays during sealing operation. Label trays with sample identification on the backside of trays. Set Quanti-Trays in 35°C incubator oven for 24 h; read results at that time.

Analytical Procedure

Read MPN results with a partner so that agreement is achieved on color determination endpoints. Colilert MPN trays yield yellow indicator scores for total coliforms and bright blue-white fluorescent scores under black-light illumination for *E. coli*. Enterolert MPN trays yield bright blue-white fluorescent scores under black-light illumination for *Enterococci*. Record MPN scores in lab notebook.

Equipment Notes

The Bio Rad Fluor S Max imager will be set up in the boathouse staff office onshore at GBF to yield a stable platform for photos. Instrument manual is located in the photocompartment of instrument. Institutional autoclaves for agar sterilization are located in Rm 503 MLML. Steam autoclave temperature is factory set; sterility of plates will be empirically ensured by preparing plates one week in advance of ballast testing and monitoring for contaminating colonies. Only use plates that appear clear after 1 week hold time.

SOP 26 Indicator Microbes *Vibrio Cholerae* Serotype 01 and *Vibrio Cholerae* Serotype 0139 for Organisms <10 µm

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Water quality indicator microbes *Escherichia coli* and *Enterococci* are to be enumerated using commercially available MPN kits from Idexx, Inc. The Colilert® kit provides MPN estimates of total coliforms and *E. coli*; Enterolert® kit provides MPN estimates of *Enterococci*. Both kits are based on species-specific chromogenic reactions that can be visualized with the naked eye and/or handheld blacklight illumination.

Method References

EPA/ETV Generic Protocol for the Verification of Ballast Water Treatment Technologies, v4.2 (2010).

Sampling Procedures

Acid wash collection carboys before each ballasting operation. Subsample three replicate 100 mL volumes into sterilized plastic sample bottle (Idexx) for each microbe container collection; 9 replicates for uptake, 9 for control, 9 for treatment. Prepare Colilert and Enterolert Quanti-Trays as per instructions supplied with each Idexx packaging box. Make sure the heated Quanti-Tray sealer has free bench space for insertion and exit of trays during sealing operation. Label trays with sample identification on the backside of trays. Set Quanti-Trays in 35°C incubator oven for 24 h; read results at that time.

Analytical Procedure

Read MPN results with a partner so that agreement is achieved on color determination endpoints. Colilert MPN trays yield yellow indicator scores for total coliforms and bright blue-white fluorescent scores under black-light illumination for *E. coli*. Enterolert MPN trays yield bright blue-white fluorescent scores under black-light illumination for *Enterococci*. Record MPN scores in lab notebook.

SOP 27 FDA-Based, Flow Cytometric Analysis of Viable Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Viable organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$ (hereafter referred to as ≥ 10 to $< 50 \mu\text{m}$) are difficult to enumerate quantitatively because live and dead cells in this size class cannot be distinguished visually in their natural state. Therefore, we have assembled several independent methods, based on culture methods and/or viability stains, that attempt to enumerate viable organisms in the ≥ 10 to $< 50 \mu\text{m}$ size class to meet the demands of ballast regulations that specifically call for numeric 'live' counts.

Here we describe a flow cytometric estimate of living cells in the size range ≥ 10 to $< 50 \mu\text{m}$ using FDA as a cellular tag for viable cells (Geary et al. 1997; Hayakawa et al. 2008). FDA is a colorless reagent that freely passes through cell membranes and, when acted upon by living cellular esterase activity, is converted to the brilliant green fluorescent product, fluorescein, which readily marks viable cells for flow cytometric detection.

Method References

Geary, S., G. Ganf, J. Brookes. 1997. The use of FDA and flow cytometry to measure the metabolic activity of the cyanobacteria, *Microcystis aeruginosa*. Verh. Int. Verein. Limnol. **4**: 1-3.

Hayakawa, M., K. Suzuki, H. Saito, K. Takahashi, and S. Ito. 2008. Differences in cell viabilities of phytoplankton between spring and late summer in the northwest Pacific Ocean. J. Exp. Mar. Biol. Ecol. **360**: 63-70.

Sampling Procedures

Ambient concentrations of living organisms in the ≥ 10 to $< 50 \mu\text{m}$ size range may be ca. 10^2 - 10^3 mL^{-1} in uptake and control water; that concentration will certainly be less in the treated samples. At this time, the maximum flow rate of most flow cytometers, including our Accuri C6 flow cytometer, is $60 \mu\text{L min}^{-1}$; thus, sample water may be concentrated to achieve reasonable count rates for cytometric analysis. If needed, we will use a combination of filter fractionation and cytometric calibration beads to isolate (physically and analytically) the ≥ 10 to $< 50 \mu\text{m}$ organisms on our flow cytometer.

Prepare ca. 100 mL particle-free seawater from the uptake source water using a $0.22 \mu\text{m}$ membrane syringe filter. The filtered water does not need to be sterile if prepared on the same day as the cytometric analysis.

The natural sample water will be sieved through a coarse filter ($73 \mu\text{m}$) to remove large particles that can clog the optical sensing zone of the flow cytometer. We use a coarse filter to allow chain-forming protists and pennate diatoms to be included in the cytometric analysis (these organisms have minimum dimensions $< 50 \mu\text{m}$ but would be removed from analysis by the $35 \mu\text{m}$ filter commonly employed in ballast-related zooplankton sampling).

Samples should be analyzed without concentration to evaluate numeric levels of organisms. If necessary, prepare concentrated samples in the following manner. Load a filter funnel with a 10 μm , 25mm diameter Nitex filter, place a 73 μm sieve over the open filter cup and draw 100 mL sample water with gentle suction through the 10 μm filter. Place the 10 μm filter in a new cytometer counting tube, add 1 mL 0.22 μm filtered seawater (from #2 above), and vortex to resuspend particles; this constitutes a 100-fold increase in the concentration of the ambient organisms from uptake and control samples. Hopefully, the majority of the smaller, but more numerous, bacteria-sized microbes will have been eliminated, resulting in less noisy cytometric detection results. This same procedure should be used for treatment samples, but with a larger 1 L sample filtration so that a 1000-fold concentration is achieved. The final volume of 1 mL will be adequate for all cytometric analyses.

Analytical Procedures

Warm up the Accuri C6 cytometer according to the instructions posted on the inside of the instrument lid. This will purge the system, establish constant flow and ready the instrument for sample introduction. Run the premixed blue laser calibration beads and ensure that the CV of the largest fluorescent peak is <5%. The instrument is ready to go.

Set up two-dimensional density plots (4 total); two with side-scatter on the x axis; two with forward-scatter on the x axis. Configure two of the forward/side scatter pairs with red fluorescence on the y axis; two with green fluorescence on y axis. (This pre-set cytogram configuration can be found under 'ballast-FDA1', but you should practice setting this up manually at least once). Set the flow rate to high (60 $\mu\text{L min}^{-1}$) and run the premixed size standards containing red fluorescent 10 μm and 50 μm beads; these populations, on forward-scatter, provide approximate lower and upper bounds for the relevant particles falling in the ≥ 10 to <50 μm category. Record the mean population values for forward scatter response from the 10 and 50 μm calibration beads.

Briefly vortex the 1 mL concentrated sample (without FDA added) and begin sample flow on the cytometer. Particle events appearing roughly within the bounds of the forward-scatter calibration size limits provide likely indications of organisms falling in the ≥ 10 to <50 μm size category. High intensity red fluorescent events are good indications of phytoplankton per se. Both autotrophs and heterotrophs will score weak, but measurable green fluorescence. Our experience suggests that target organisms will form sharper population clusters in side scatter view, than in forward scatter view (but remember, forward scatter is generally expected to be a better scaler for size than side scatter). Use the computer region tool to graphically define the entire size fractionated collection of population events. Depending on species assemblages, this group may appear as multiple populations or a single large cluster. This region will be used to define a gate that is turned off and on between data file accumulations. Our experience suggests that, at times, this gate can be useful in reducing noise after FDA-staining that might arise from viable particles <10 μm (presumably bacteria) present in the filtered seawater preparation in which the ≥ 10 to <50 μm particles are suspended.

Once the gate is established, run the sample, without FDA added, for 2 min with the gate toggled on and then for 2 min. with the gate toggled off. Add FDA (in DMSO working stock) to yield a final concentration of 10 μM , vortex briefly and immediately run the

sample for 2 min., once with the gate toggled on, again with the gate toggled off. Wait 5 min., briefly vortex the sample and repeat the samples runs, again with the gate toggle on, and then toggled off. You should collect two files without FDA and four files with FDA; half with the gate toggled on, half with the gate toggled off.

To determine the number of viable cells, draw a rectangular region above the original gate, defined before adding FDA, bounded on the left and right by the approximate position of the 10 μm and 50 μm size calibration beads. Determine the number of particles that move up from the original location into the region of higher green fluorescence. This is the viable cell count. Determine the viable cell concentration from the cytometric flow rate, sample collection time and concentration factor used in preparing the 1 mL sample volume (nominally, 100x for uptake and control; 1000x for treatment).

Following organism analysis, two separate samples will be preserved in 1% glutaraldehyde and stored for at least six (6) months.

Equipment Notes

Start-up/shut-down instructions for the Accuri C6 flow cytometer are given in the instrument manual stored in the organizer compartment under the lid of the instrument.

SOP 28 FDA/CMFDA Epifluorescence Analysis of Viable Organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Viable organisms $\geq 10 \mu\text{m}$ but $< 50 \mu\text{m}$ (hereafter referred to as ≥ 10 to $< 50 \mu\text{m}$) are difficult to enumerate quantitatively because live and dead cells in this size class often cannot be distinguished visually. Therefore, we have assembled several independent methods, based on culture methods and/or viability stains, that attempt to enumerate viable organisms in the ≥ 10 to $< 50 \mu\text{m}$ size class to meet the demands of ballast regulations that specifically call for numeric ‘live’ counts.

Here we describe an epifluorescence microscopic analysis of living cells in the size range ≥ 10 to $< 50 \mu\text{m}$ using a combination of fluorescein diacetate (FDA) and chloromethylfluorescein diacetate (CMFDA) as cellular tags for viable cells (Drake et al. 2010). FDA and CMFDA are colorless reagents that freely pass through cell membranes and, when acted upon by living cellular esterase activity, are converted to the green fluorescent products, fluorescein and chloromethylfluorescein, which readily mark viable cells for epifluorescence detection. The larger molecular size of CMFDA has been observed to increase the retention time of fluorescein based product within the cell interior.

Method References

EPA/ETV Generic Protocol for the Verification of Ballast Water Treatment Technologies, v4.2 (2010).

Drake, L.A., M.K. Steinberg, S.H. Robbins, S.C. Riley, B.N. Nelson and E.J. Lemieux. 2010. Development of a method to determine the viability of organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ (nominally protists) in ships’ ballast water: a combination of two vital, fluorescent stains. Letter report submitted to the Naval Research Laboratory.

Sampling Procedures

Ambient concentrations of living organisms in the ≥ 10 to $< 50 \mu\text{m}$ size range may be ca. 10^2 - 10^3 mL⁻³ in uptake and control water; that concentration will certainly be less in the treated samples. The method of Drake et al (2010) utilizes a 1 mL Sedgewick Rafter counting chamber for visual epifluorescent enumeration of live cells marked by green fluorescence and native motility. The uptake and control samples are expected to yield an adequate number of live counts (> 100) if the entire counting chamber is examined. This can be accomplished using a 10x objective (total 125x power on our Zeiss epifluorescence microscope) which will provide adequate working distance to examine the full 1mm depth of the counting chamber while at the same time providing adequate magnification to visualize particles in the ≥ 10 to $< 50 \mu\text{m}$ range.

The treatment sample will be concentrated by three-fold if numeric observations of live cells are low. Load an ‘inner cylinder’ unit with a 10 μm Nitex Filter, and gently push through the unconcentrated 1 L sample to remove 666 mL of ‘filtered water’ resulting in a three-fold

final concentration of original sample. Lift ‘inner filter cylinder’ several times to back-wash organisms from Nitex screen.

Analytical Procedure

Add FDA and CMFDA (working stock dissolved in DMSO) to the sample to yield final concentrations of 5 μ M and 2.5 μ M, respectively. Shake gently and immediately load the counting chamber free of bubbles. Under blue excitation/green emission, scan the sample using gridlines to track the full volume of the counting chamber noting green fluorescent cells and motile cells as viable counts. Work fast, as the green fluorescent tag may show considerable fade within 15 min. Ignore live cells $\geq 50 \mu$ m in minimum dimension based on rapid visual size assessment.

Following organism analysis, two separate samples will be preserved in 1% glutaraldehyde and stored for at least six (6) months.

Equipment Notes

Use the blue excitation FITC optical cube aligned in the Zeiss Standard epifluorescence microscope under 50W mercury lamp excitation. Check ocular counting grids with stage micrometer to verify 10 μ m and 50 μ m size limits.

SOP 29 In-situ (Thermosalinograph (TSG) Operation, Data Collection and Data Processing

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Shaft Alley

- Ensure after ballast pump discharge TSG supply valve Q1 on 8-inch pipe is fully open.
- Ensure ballast pump suction TSG return valve Q2 on 8-inch pipe is fully open.
- Ensure debubbler/manifold inlet valve Q3 is closed.
- Ensure debubbler/manifold air vent valve Q4 is closed.
- Ensure debubbler/manifold seawater discharge ball valve Q5 is open.
- Ensure debubbler/manifold seawater to TSG valve Q6 and Q7 are open.
- Ensure debubbler/manifold seawater from TSG valve Q8 and Q9 are open.
- After system circulation (water flow) has started, adjust debubbler/manifold valve TSG Q5 so that a flow rate of between 10 and 15.8 gallons per minute is obtained.
- Switch on Sea-Bird TSG Interface Box; red power light indicator should be on.

Tech Library

- On IMAC server, start SEASAVE data acquisition software to collect data at a frequency of six-times per minute.
- Use the same test naming format for the for data file.

Shaft Alley

- On completion of test, i.e. when water flow through the pipes has stopped, stop data acquisition via SEASAVE.
- Turn off power to Sea-Bird TSG Interface Box.
- Secure valves to and from TSG.

Tech Library

- Start SEASAVE Processing software script, SEAPROC, to create one-minute averaged data files using the same naming format and store in appropriate directory.
- Store all files in a digital format and store in appropriate directory.
- Scan all TSG instrument calibration files and store in digital format and store in appropriate directory.

SOP 30 In-situ Probe Measurements: Dissolved Oxygen, Conductivity, Salinity, Temperature, pH

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

A hydrographic monitoring system will be used to measure dissolved oxygen, turbidity, salinity and temperature in the biological sampling tanks at the time of sample collection. In the lab, a benchtop pH meter with Ross combination electrode will be used to measure pH. Some of the measurements made here are redundant with those collected continuously by independent sensors associated with the GBF data logging system.

Method References

EPA Method 360.1 (Probe Method)

Sampling Procedures

A YSI 6-Series environmental monitoring system will be immersed in the biological sampling tanks to measure dissolved oxygen, turbidity, conductivity, and temperature (salinity is derived from the conductivity and temperature data). An aliquot of the same sample water will be used for pH measurement using a Beckman Model 73 pH meter with ThermoOrion Ross combination electrode.

Analytical Procedures

The YSI monitoring system's oxygen sensor will be calibrated per manufacturer's instructions in a special air saturated compartment located on the instrument. Conductivity and temperature is factory calibrated and will be checked at least once per year or between manufacturer tests. Temperature response will be checked with a manual thermometer and turbidity will be calibrated with appropriate standard according to manufacturer's instructions. This procedure will be repeated for each ballasting sequence (nominally, once per week).

pH is measured after a two point buffer calibration (pH 10, pH 7) according to instrument instructions attached to the Beckman pH meter. 20 mL seawater samples will be measured in triplicate.

Equipment Notes

Beckman pH meter model 71. Instructions are fixed to the instrument in slide out sheets to perform two point buffer calibration. Use dated Fisher calibration buffers pH 7 and pH 10.

SOP 31 Grab Sample: Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON)

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Particulate Organic Carbon (POC) is determined by combustion analysis on a CEC 440 CHN Analyzer, maintained and operated at MLML. Samples are collected on precombusted Whatman GF/F filters, dried and packed in pre-combusted, tin drop-capsules for automated sample loading.

Quantitative estimates of organic carbon (POC) are calculated using acetanilide as a standard.

Method References

EPA Method 415.3

Sampling Procedures

- Clean filter funnels and plastic sample containers with hot tap water and nylon bottle brush; rinse with filtered Nanopure water. Wipe stainless steel filter forceps with methanol or acetone, prior to filter handling. Avoid handling glass fiber filters with bare hands; POC from skin oils can contaminate filters and labware. Whatman GF/F 25mm filters should be pre-combusted at 450 °C for one hour in a muffle furnace; keep filters in closed containers thereafter.
- Filter water samples volumetrically under vacuum not to exceed ca. one-third an atmosphere (ca. 25mm Hg); triplicate samples should be processed. POC analysis requires visible coloration from particulates on filter for ample sensitivity; nominally, a 280mL filtration of SF Bay water should be adequate. Keep filtration times for each sample <15min by adjusting volumes accordingly; record volumes for each sample.
- Fold each sample glass fiber filter in half, using two forceps. Place filters in plastic film envelopes; all replicates of equal filtration volume can be placed in the same envelope. Mark envelope with date, sample name and filtration volume, place in drying oven (65 °C) for 48 hours. Transfer to vacuum dessicator and hold at room temperature, under vacuum until instrumental analysis.

Analytical Procedures

- The ballast sampling team will be responsible for preparing POC filters for automated sample injection; after preparation, the samples will be delivered to MLML for analysis.
- Tin drop-capsules must be combusted for one hour at 450 °C to remove manufacturing contaminants. Combusted tin capsules can be stored in a closed container and place in a vacuum dessicator for use as necessary.
- Prepare samples on a dust-free plastic cutting sheet (as found in kitchen stores); all plasticware, forceps, well-plates, etc. must be cleaned and dried prior to filter packing operations.

- Load tin capsules into flexible, plastic 96-well plates as necessary. Using two stainless steel forceps, fold POC filter in multiple bends retaining triangular shape (like a coffee filter); this is accomplished using gentle force on the clean plastic working surface. Insert filter into tin capsule and force all filter material into the interior of the capsule; no filter edges can be left outside the open top of tin capsule (this will foul the gravity-drop autosampler system).

Place each completed sample in a marked well of the final 96-well plate; this will serve to store and organize sample filters just prior to analysis. Caution: use care not to bump trays; always cap with a lid. Record sample identity/tray locations for numerically sequenced autosampler loading. Tape lid with short pieces of labeling tape; deliver to MLML CHN Room 512, store in dessicator.

SOP 32 Grab Sample: Total Suspended Solids (TSS)

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Total suspended solids are determined gravimetrically on dried, pre-weighed 47mm glass fiber filters (1.5 μ m nominal pore size). The pre-weighed filters are commercially available as Proweigh™ filters (Environmental Express, Inc., part no. F93447MM). Final gravimetric weights are determined on a calibrated balance with 0.01 mg resolution.

Method References

Standard Methods 2450 D, 20th Edition

EPA Method 160.2

Sampling Procedures

Filter water samples volumetrically under vacuum not to exceed one-third atmosphere (e.g., 25 cm Hg; 10 in Hg; 5 psi); triplicate samples should be processed. The anticipated TSS load in the Carquinez Straits is expected to be >20 mg/L; the tare weight of each filter is roughly 120 mg. Thus, it is desirable to filter as much water as possible to yield a final TSS weight that is significantly higher than the filter tare weight. Start by filtering 280 mL using volumetrically-calibrated, clean polybottles filled to the brim. Quantitatively add more volume such that the total filtration time does not exceed 30 min for each filter. Nominally, you should be able to filter at least 560 mL unless extremely turbid conditions exist. Record total volumes filtered for each sample on the filtration data logging sheet.

Place completed TSS filter back into its numbered tray, particle side up. Set all filter trays carefully in drying oven. Set oven to 65°C for 48 hours. Place dry samples in a vacuum desiccator at room temperature until gravimetric analysis.

Analytical Procedures

All gravimetric weighings must be made at the shore lab (MLML); not on board ship. Use the Mettler balance (0.01 mg resolution) in Room 512 (MLML). Transfer the perforated desiccant container from the 65 °C drying oven in Rm. 512 to the Mettler weighing chamber at least 30 min prior to making weight determinations; this will condition the weighing chamber to a uniform humidity. Weigh filters (without aluminum tray) and record values along with marked tray number and tared filtered weight on data logging sheet. Return filters to their respective aluminum trays.

Calculate TSS concentrations using noted weights and filtration volumes. Inspect data for questionable results, e.g., >1.5-fold variation within any given set of triplicates. Re-weigh questionable samples; mark data sheet with new weight readings. Filters and trays may be disposed of at this time.

SOP 33 Grab Sample: Transmittance

Application	LAND-BASED	XX	SHIPBOARD	XX
-------------	------------	----	-----------	----

Method Overview

Transmittance is analyzed by means of placing a focused receiver at a known distance away from a light source of a specific frequency. The ratio of light gathered by the receiver to the amount originating at the source is known as the beam transmittance (Tr). This is performed at frequencies from 370 nm to 650 nm, and distances of 10 cm and 25 cm.

Sampling Procedures

Samples are collected in glass vials fit with Teflon-lined screw caps (20mL). The glass vials are acid washed and copiously rinsed with 0.22 μ m filtered deionized water (Barnsted Nanopure). Sample water is not filtered, filling the 20 mL glass vial to capacity. Three replicate sample vials will be prepared for each water sample. The samples will be stored at room temperature and analyzed within twelve hours. Three samples each of Nanopure water will be prepared as above as blanks.

Analytical Procedures

Analysis will be completed by MLML.

SOP 34 Grab Sample: Dissolved Organic Carbon (DOC)**Application****LAND-BASED****XX****SHIPBOARD****XX****Method Overview**

Particle free water is analyzed for dissolved organic carbon (DOC) by means of high temperature catalytic oxidation (Shimadzu TOC-V(CSH) Analyzer). Analyses will be contracted through McCampbell Analytical, Inc., Pittsburg CA (an EPA-approved local analytical facility 5000A Analyzer).

Method References

Standard Method 5310-B

EPA Method 415.3

Benner, R. and M. Strom. 1993. A critical evaluation of the analytical blank associated with DOC measurements by high temperature catalytic oxidation. *Marine Chemistry* 43:151-160.

Sampling Procedures

DOC samples are collected in glass vials fit with Teflon-lined screw caps (20mL). The glass vials are acid washed and copiously rinsed with 0.22 μm filtered deionized water (Barnsted Nanopure). Sample water is passed through a 0.45 μm membrane filter, filling the 20 mL glass vial to 2/3 capacity. Three replicate sample vials will be prepared for each water sample. The DOC samples will be frozen (-20°C) immediately after preparation and stored as such until analysis. Three samples each of Nanopure water, with and without 0.45 μm filters will be prepared as above to check for DOC contamination.

Analytical Procedures

DOC analysis will be completed by McCampbell Analytical, Inc., Pittsburg, Calif. (EPA approved laboratory).

Appendix A Brine Augmentation Procedure (LAND-BASED)

Application	LAND-BASED	XX	SHIPBOARD	
-------------	------------	----	-----------	--

Instructions

Person-in-Charge (PIC) shall ensure that the overall data log is accurate, and sign each log sheet as soon as it is completed. Each completed log sheet shall be submitted to the Quality Officer (Quality) as soon as the SOP is completed.

Support: If the person filling in the data is not the same as the PIC, that person shall be listed below as "Support." Each sheet that they entered data shall also bear their name, noting "data entry by" and their name.

Quality Officer (Quality) shall verify and sign each completed log sheet, scan into the online records system, and securely store in accordance with the Quality Management Plan.

Manual-hand data logs are provided for ALL test parameters. This ensures that testing may be conducted and verified even in the event of an automation system or online records system failure.

Automation system and Online forms are available for most data sheets. DO NOT make duplicate records. UTILIZE the automation system and online forms wherever possible. If part of a data log is manual-hand and part is online, hand write "ONLINE RECORD" on the log sections which are recorded online.

Person-in-Charge (Name/Title): _____

Quality Officer (Name): _____



Support (Name/Title): _____

Support (Name/Title): _____

Notes: _____

CMA – GOLDEN BEAR FACILITY UPGRADES

Brining Operations

PREPARED FOR: California Maritime Academy Vallejo, California		BY: Jake Parks PROJECT ENGINEER	
 THE GLOSTEN ASSOCIATES 1201 Western Avenue, Suite 200, Seattle, WA 98101-2921 TEL 206.624.7850 FAX 206.682.9117 www.glosten.com		CHECKED: Kevin J. Reynolds, PE PROJECT MANAGER	
		APPROVED: David Larsen, PE PRINCIPAL-IN-CHARGE	
		DATE: 26 March 2012	
DOC:	REV: B	FILE: 11097.02	

The Golden Bear Facility is required to increase the salinity of two ballast water tanks, combined 710m³ capacity, from ~15psu (depending on tide) to 32 – 35 psu for testing purposes. This will be accomplished by using a vacuum truck to pick up salt from a supplier, bring the salt to the test facility, mix it with seawater to make brine, and then pump the brine directly into the ballast tanks.

Key Contacts

Golden Bear Facility (GBF) Receives Brine	RES Environmental Services Transports Salt/Makes Brine	John's Salt Service Supplies Salt
Test facility with ballast tanks that require the brine	Salt/Brine Transportation: 3,000 gal Vacuum Truck with 375 cfm vane pump, 800 gpm mixing pump, discharge tee, and hoses	Supplier of the Bulk Dry Coarse Salt (Smaller Coarse if possible)
California Maritime Academy 200 Maritime Academy Dr Vallejo, CA 64590	RES Environmental Services 2153 Martin Way Pittsburg, CA 94565	John's Salt Service 38507 Cherry St Newark, CA 94560
Richard Muller (707) 654-1258 rmuller@csum.edu	Craig Joseph (925) 432-1755 craigjoseph@resenv.com	Shane Persiani (510) 713-2107 johnssalt@att.net

Approach/Calculations

Please reference the tables located on the last page of these instructions.

A “dose” is one vacuum truck (~3,000 gallons) of brine at between 80 and 100 Sal (defined below). An effort has also been made to ensure that the final (half) dose can vary between 80 – 100 Sal and still hit the target salinity in the ballast tanks.

The vacuum truck(s) will typically pick up enough salt in a single run for more than two doses. This means that the truck will be able to create a load of brine, and still have enough un-dissolved salt remaining for a second load of brine if needed. This eliminates at least one time consuming cross-town drive. This additional salt also speeds the dissolution of the salt.

Table 1 matches the initial measured ballast tank salinity to determine the number of doses required, the number of rented trucks required, and total salt to be provided by the salt supplier. This amount is the total for both ballast water tanks.

Table 2 specifies how much salt is to be loaded into each truck.

Table 3 is for ONE ballast tank during the brining period. After each dose of brine is added to a ballast tank, this table provides the number of additional doses required for that one tank to reach the target salinity.

Key Definitions

Sal = Degrees Sal = % Saturation

This unit is measured with a salinometer and ranges from 0 – 100 degrees.

PSU = Practical Salinity Unit

This unit is measured by an electrical conductivity ratio and ranges from 0 to 264 for saltwater brine. YSI Sonde will display PSU as Sal.

% Salt = Percent Salt by weight (lb/gal) = $\text{PSU} \times 10$

This unit cannot be measured directly.

Dose = 3,000 gallons of brine at 80 Sal (~6,000 lb of salt)

Half Dose = 1,500 gallons of brine at 80 Sal **or** 3,000 gallons of brine at 43 Sal (~3,000 lb of salt)

Logistics Checklist

- ☐ **GBF** initially fills the ballast water tanks, measures the salinity in the tanks, calculates the required additional salt, and places a delivery order with RES Environmental. This might be one truck that will split its load between the two ballast tanks, or two trucks each dedicated to a single tank.

Fill ballast tanks to ~85% capacity (620 m³) [164,000 gal] and measure salinity in psu.

Port (psu)	Starboard (psu)	(Port×Starboard)/2 = Average (psu)

Use **Table 1** to determine how much salt and how many trucks will be needed.

Average (psu) = Tested Salinity (psu)	Trucks Needed	Total Salt Needed (lbs)

Place order with Salt/Brine Transport. Deliver no later than _____

- ☐ **RES**, after receiving order from GBF, places a salt order with John's Salt. RES prepares the required number of vacuum trucks with special pumps, mixing nozzles, and hoses. RES picks up the required salt from John's Salt.

Truck 1 Salt (lbs)	Truck 2 Salt (lbs)	Truck 3 Salt (lbs)	Total Salt (lbs)

- ☐ **John's Salt**, after receiving order from RES, loads requested quantity of salt into RES truck(s).
- ☐ **GBF** ensures ballast tank mixing systems are running during brine delivery and provides a diffuser net for RES discharge hose.
- ☐ **RES** delivers the truck(s) of salt to GBF. RES vacuums seawater into truck(s) from the GBF dock. RES uses special mixing rig to create brine of at least 80° Sal. RES pumps the requested dose (half or one) of brine, **AVOIDING UNDISSOLVED SALT**, through a diffuser net into GBF ballast tank(s) over a period of at least one hour per dose.
- ☐ **GBF** measures salinity in each ballast tank after each dose of brine. GBF informs RES how many additional doses of brine are required per tank: none, half dose, or one dose.
- ☐ **RES** repeats the vacuum, mixing, and pumping of the required doses (half or one) as required.
- ☐ **GBF** Once tanks have both achieved target salinity of 32-35 PSU, now "tops off" the ballast tanks with seawater and the brining operation is complete.

Reference Tables

Table 1
Initial Ballast Tank Salinity, Brine
Requirements (Both Tanks)

Ballast Tank Salinity (psu)	80 Sal Doses Needed (No.)	3k gal Truck Needed (trucks)	Total Salt Needed (lbs)
35	0	0	0
34	0	0	0
33	0	0	0
32	0	0	0
31	1	1	7,000
30	1	1	7,000
29	2	1	14,000
28	2	1	14,000
27	2	1	14,000
26	2.5	2	21,000
25	2.5	2	21,000
24	2.5	2	21,000
23	3.5	2	28,000
22	3.5	2	28,000
21	4	2	28,000
20	4	2	28,000
19	4	2	28,000
18	5	3	35,000
17	5	3	35,000
16	5	3	35,000
15	5.5	3	42,000
14	5.5	3	42,000

Table 2
Salt Required for Each Truck

Truck No. 1 (lbs)	Truck No. 2 (lbs)	Truck No. 3 (lbs)
-	-	-
-	-	-
-	-	-
-	-	-
7,000	-	-
7,000	-	-
14,000	-	-
14,000	-	-
14,000	-	-
14,000	7,000	-
14,000	7,000	-
14,000	7,000	-
14,000	14,000	-
14,000	14,000	-
14,000	14,000	-
14,000	14,000	7,000
14,000	14,000	7,000
14,000	14,000	7,000
14,000	14,000	14,000
14,000	14,000	14,000

Table 3
Required Brine for
One Ballast Tank

Tested Tank Salinity (psu)	80 Sal Doses Needed (No.)
35	0
34	0
33	0
32	0
31	0.5
30	0.5
29	1
28	1
27	1
26	1
25	1
24	1
23	1.5
22	1.5
21	2
20	2
19	2
18	2.5
17	2.5
16	2.5
15	2.5
14	2.5

Handy Calculations

1,500 gal @ 96 deg Sal = 3,783 lb Salt = 4.8 PSU rise in single ballast tank.

1,500 gal @ 81 deg Sal = 3,105 lb Salt = 3.4 PSU rise in single ballast tank.

Truck tank mixing takes about 20 minutes to reach 80 degrees Sal or greater.

It takes roughly 1 hour to perform a half dose (1,500 gallons @ 80 deg Sal) brine cycle.

Appendix B BWMS Maintenance Log

Golden Bear Facility Maintenance Log

Trojan Ballast Water Management System

IMO G8 Shipboard Testing

Date	Test	Maintenance Task	Type of Maintenance
1-May-2012	2	A complete filter change was conducted by the BWMS manufacturer between shipboard Test 1 and Test 2. BWMS R&D trials between shipboard Tests 1 and 2 caused a failure at the filter's seam welds. The original filter was replaced with a new filter with the same specifications but improved weld seam construction. The new filter series was marked 1-12 "B" to differentiate from the initial set which had no letter designation.	Repair / Preventive
7-Oct-2012	3	On initial start-up of BWMS in preparation for uptake, an alarm occurred for UV lamp Position 32. The BWMS manufacturer inspected UV lamps, and the UV lamp connector was detached and re-attached as a condition check prior to re-starting the BWMS. No alarm occurred upon BWMS re-start and shipboard Test 2 continued as planned.	Repair
7-Oct-2012	3	A complete filter change was conducted by the BWMS manufacturer between shipboard Test 2 and Test 3. The filter change was prompted by events outside the scope of shipboard testing or operations. It is suspected that the filters were compromised during an extremely high differential pressure spike that occurred as the 250i discharge valve was opening after the warm-up period due to system start-up issues. Following this event, modifications were made to the start-up sequence (see e-mail from Bill Davidson to Jad Mouawad dated 13 August 2012 more details). After inspection it was determined that the filters should be replaced as a precaution. The replacement filters were reported as the same specification and weld seam construction as the previous filters. Twelve (12) new filters were marked numerically 13-19 and 21-25 to differentiate from the previous set.	Preventive
7-Oct-2012	3	UV lamp in Position 11 was replaced with a spare by the vessel's engineer on BWMS start up at sea during a treated ballast water exchange. The UV lamp failure was indicated on the BWMS control panel and was the only lamp replaced during the project.	Repair
20-Mar-2013	4	A complete filter change was conducted by the BWMS manufacturer between shipboard Test 3 and 4. The new filters installed were manufactured with the final production material, a Super Duplex/Hastelloy blend, and are of the same specification and manufacturer as the previous 316 Stainless Steel prototype filter elements. The BWMS manufacturer requested that these final production filters be tested in the additional shipboard test to confirm treatment efficacy. Twelve (12) new filters were marked numerically 26-37 to differentiate from the previous set.	Preventive

Appendix C Project Training Records

Golden Bear Facility Training Log
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

	Dave Coleman	Mick Bowlin	John Coyle	Bill Davidson	James Eckerle	Matt Hobbs	Dan Lintz	Richard Muller	Nic Shields	Bill Schmidt	Dan Weinstock
Standard Operating Procedures (SOPs) for Golden Bear Facility Operations											
SOP 1 - Quality Control Checklist for Project				1-Jan-10				1-Jan-10			
SOP 2 - Posted Placard and Piping Line-ups (LB)				1-Jan-10				1-Jan-10			
SOP 3 - Posted Placard and Piping Line-ups (SB)				1-Jan-10				1-Jan-10			
SOP 4 - BWTS Commissioning Data Sheets				1-Jan-10				1-Jan-10			
SOP 5 - BWMS Shakedown Test Data Sheets				1-Jan-10				1-Jan-10			
SOP 6 - Quality Control Checklist for Cycle (LB)				1-Jan-10				1-Jan-10			
SOP 7 - Quality Control Checklist for Cycle (SB)				1-Jan-10				1-Jan-10			
SOP 8 - Tank Cleaning (LB)				1-Jan-10				1-Jan-10			
SOP 9 - Tank Cleaning (SB)				1-Jan-10				1-Jan-10			
SOP 10 - Preparation of Source Tanks	1-Jan-13			1-Jan-10				1-Jan-10	1-Sep-11		
SOP 11 - Calibration of Sampling System Flow Meter				1-Jan-10				1-Jan-10	1-Sep-11		
SOP 12 - Calibration of Sea-Bird Thermosalinograph (TSG)				1-Jan-10				1-Jan-10			
SOP 14 - Operation of BWMS and Facility Piping System (LB)	1-Jan-10	1-Oct-12	1-Oct-12	1-Jan-10			1-Jan-10	1-Jan-10	1-Sep-11	1-Jan-10	1-Jan-10
SOP 15 - Operation of BWMS and Facility Piping System (SB)	1-Jan-10	1-Oct-12	1-Oct-12	1-Jan-10			1-Jan-10	1-Jan-10	1-Sep-11	1-Jan-10	1-Jan-10
SOP 16 - Operation of Ballast Water Sampling System (LB)		1-Oct-12		1-Jan-10		1-Mar-12		1-Jan-10			
SOP 17 - Operation of Ballast Water Sampling System (SB)		1-Oct-12		1-Jan-10		1-Mar-12		1-Jan-10			
SOP 18 - Chain of Custody				1-Jan-10				1-Jan-10			
SOP 29 - In-situ TSG Operation, Data Collection and Data Processing				1-Jan-10				1-Jan-10	1-Sep-11		

LB = land-based; SB = shipboard

**Golden Bear Facility Training Log
Trojan Ballast Water Management System
IMO G8 Shipboard Testing**

	Nick Welschmeyer	Brian Maurer	Julie Kuo	Nicole Bobco	Kristin Meagher	Michelle Maraffini	April Woods	Catherine Drake	Marilyn Cruikshank	Heather Fulton-Bennett	Liz Lam
Standard Operating Procedures for Science Analysis											
SOP 13 - Calibration of Flow Cytometer	1-Jan-09	1-Jan-10	1-Sep-10								
SOP 13 - Calibration of Lab Fluorometer	1-Jan-09	1-Jan-10	1-Jan-10	1-Nov-10			1-Jan-12			1-Jan-12	
SOP 20 - Poke and Probe Viability Determination for Organisms ≥50 µm	1-Jan-09	1-Jan-10	1-Jan-10	1-Nov-10	1-Mar-11	1-Mar-11	1-Jan-12	1-Jan-12	1-Jan-12	1-Jan-12	1-Feb-12
SOP 21 - Most Probable Number (MPN) Determination of Viable Phytoplankton Cells ≥10 to <50 µm, Chlorophyll-based	1-Jan-09	1-Jan-10									
SOP 22 - C-14 Primary Production	1-Jan-09	1-Jan-10									
SOP 23, 31, 32, and 34 - Filtration; Chl α , POC, TSS, DOC	1-Jan-09	1-Jan-10	1-Jan-10	1-Nov-10	1-Mar-11	1-Mar-11	1-Jan-12	1-Jan-12	1-Jan-12	1-Jan-12	1-Feb-12
SOP 24 - Heterotrophic Bacteria Plate Counts for Organisms <10 µm	1-Jan-09	1-Jan-10	1-Jan-10								
SOP 25 - Indicator Microbes <i>E. coli</i> and <i>Enterococci</i> for Organisms <10 µm	1-Jan-09	1-Jan-10	1-Jan-10	1-Nov-10	1-Mar-11	1-Mar-11	1-Jan-12	1-Jan-12	1-Jan-12	1-Jan-12	1-Feb-12
SOP 26 - Indicator Microbes <i>Vibrio cholerae</i> Serotype 01 and <i>Vibrio cholerae</i> Serotype 0139 for Organisms <10 µm	1-Jan-09	1-Jan-10	1-Jan-10								
SOP 27 - FDA-Based, Flow Cytometric Analysis of Viable Organisms ≥10 µm but <50 µm	1-Jan-09	1-Jan-10									
SOP 28 - FDA/CMFDA Epifluorescence Analysis of Viable Organisms ≥10 µm but <50 µm	1-Jan-09	1-Jan-10	1-Jan-10								
SOP 30 - In-situ Probe Measurement; pH	1-Jan-09	1-Jan-10	1-Jan-10	1-Nov-10							
Other - Scan Biological Datasheets	1-Jan-09	1-Jan-10	1-Jan-10	1-Nov-10	1-Mar-11	1-Mar-11	1-Jan-12	1-Jan-12	1-Jan-12	1-Jan-12	1-Feb-12
Other - Scan Biological Notebooks	1-Jan-09	1-Jan-10	1-Jan-10				1-Jan-10				
Other - UV-T Analysis	1-Jan-09	1-Jan-10									

Appendix D Ballasting Operations Log

TANK ACTIVITY RECORD

TANK NAME: SWB 3-154-1

HEIGHT:

TYPE: BALLAST

CAPACITY:

gal / 1140zz LT 436

DATE	ACTION	AMOUNT		FILL SOURCE	SPECIFIC GRAVITY	DISCHARGE TO	POSITION		REMARKS
		Gallons	Tons				Lat.	Long.	
1/25/11	BALLAST	114022	425	CARGO STRTS.	1.010		CM 17	PIER	USING HYDE TREATMENT SYS.
1/27/11	DE-BALLAST	114022	425	CARGO STRTS.	1.010	CARGO STRTS.	CM 17	PIER	USING HYDE TREATMENT SYS.
3/17/12	UPTAKE	54519	208	S.F. BAY	1.010		SF.	BAY	TROSAN SB TEST
3/22/12	DISCH.	52944	202	S.F. BAY	1.010	CARGO STRTS.	CM 17	PIER	TROSAN S.B. TEST
5/01/12	UPTAKE	59234	226	S.F. BAY	1.010		S.F.	BAY	TROSAN S.B. TEST.
5/06/12	DISCH	57662	220	S.F. BAY	1.010	SEA			TROSAN S.B. TEST.
8/30/12	UPTAKE	54236	205	3-174-1	1.025		CM 17	PIER	TROSAN L.B. TEST
9/04/12	DISCH	54236	205	3-174-1	1.025	CARGO STRTS	CM 17	PIER	TROSAN L.B. TEST
10/07/12	UPTAKE	52158	199	S.F. BAY	1.010		ANCH.	#7	TROSAN SB TEST
10/12/12	DISCH	51110	195	S.F. BAY	1.010	CARGO STRTS.	CM 17	PIER	TROSAN SB. TEST
10/17/12	UPTAKE	55041	213	3-174-1	1.010		CM 17	PIER	TROSAN L.B. TEST
10/22/12	DISCH	55041	213	3-174-1	1.010	CARGO STRTS	CM 17	PIER	TROSAN L.B. TEST
3/20/13	UPTAKE	55303	211	S.F. BAY	1.010		ANCH.	#7	TROSAN S.B. TEST
3/25/13	DISCH	53993	206	S.F. BAY	1.010	CARGO STRTS.	CM 17	PIER	TROSAN S.B. TEST.

TANK ACTIVITY RECORD

TANK NAME: SWB. 3-154-2

HEIGHT:

TYPE: BALLAST

CAPACITY:

gal / 116378 LT 445

DATE	ACTION	AMOUNT		FILL SOURCE	SPECIFIC GRAVITY	DISCHARGE TO	POSITION		REMARKS
		Gallons	Tons				Lat.	Long.	
1/25/11	BALLAST	112728	420	CARQ. STRTS	1.010		CM/A	PIER	USING HYDE TREATMENT SYS.
1/27/11	DE-BALLAST	112728	420	CARQ. STRTS	1.010	CARQ. STRTS	CM/A	PIER	USING HYDE TREATMENT
3/17/12	UPTAKE	55565	212	S.F. BAY	1.010		S.F.	BAY	TROJAN S.B. TEST
3/22/12	DISCH	54255	207	S.F. BAY	1.010	CARQ. STRTS	CM/A	PIER	TROJAN S.B. TEST
5/01/12	UPTAKE	54517	208	S.F. BAY	1.010		S.F.	BAY	TROJAN S.B. TEST
5/06/12	DISCH	53206	203	S.F. BAY	1.010	SEA			TROJAN S.B. TEST
8/30/12	UPTAKE	53469	202	3-174-2	1.025		CM/A	PIER	TROJAN L.B. TEST
9/04/12	DISCH	53469	202	3-174-2	1.025	CARQ. STRTS	CM/A	PIER	TROJAN L.B. TEST
10/07/12	UPTAKE	52681	201	S.F. BAY	1.010		ANCH.	#7	TROJAN S.B. TEST
10/12/12	DISCH	51634	197	S.F. BAY	1.010	CARQ. STRTS	CM/A	PIER	TROJAN S.B. TEST
10/17/12	UPTAKE	55827	213	3-174-2	1.010		CM/A	PIER	TROJAN L.B. TEST
10/22/12	DISCH	55827	213	3-174-2	1.010	CARQ. STRTS	CM/A	PIER	TROJAN L.B. TEST
03/20/13	UPTAKE	54517	208	S.F. BAY	1.010		ANCH.	#7	TROJAN S.B. TEST
03/25/13	DISCH	53206	203	S.F. BAY	1.010	CARQ. STRTS	CM/A	PIER	TROJAN S.B. TEST

TANK ACTIVITY RECORD

TANK NAME: 3-174-1

TYPE: SWB

HEIGHT: 26' 0 1/4"

CAPACITY: 92064 gal / 351 LT (SW)

DATE	ACTION	AMOUNT		FILL SOURCE	SPECIFIC GRAVITY	DISCHARGE TO	POSITION		REMARKS
		Gallons	Tons				Lat.	Long.	
5/14/06	DEBALLAST	51801	193	VALLEJO MUNI	1.000	SEA			
6/29/06	BALLAST	92064	343	VALLEJO MUNI	1.000		AT VALLEJO, CA		FILL OVER TOP
4/23/07	DEBALLAST	92064	343	— " —	1.000	CARG. STRTS.	CMA PIER		CRU 1 2007
July 3, 09	BALLAST DEP	72736	271	Vallejo Muni	1.000	" "	CMA PIER		
August 4, 09	Deballast	28182	105		1.000	SEA	21-56N	148-52W	
8-11-09	Ballast AND	43388	166	Ocean	1.025		20-11N	157-54W	Trim
8/19/09	Deballast	43492	166		1.025	SEA	24-30N	140-42W	Ballast Exchange
4-28-10	Ballast	62642	239	Carg straits	1.025		CMP	Pier	Trim
5-10-10	Deballast	62642	239	Ocean	1.025	SEA	42-00N	169-41.2W	Ballast Exchange
5-10-10	Ballast	62642	239	Ocean	1.025	SEA	42-00N	169-41.2W	" " Traded
5-19-10	DE BALLAST	62642	239		1.025	SEA	39-07N	136-26E	DEBALLAST FOR TRIM
5/26/10	BALLAST	41,315 KAS	43.2 KAS	OCEAN	1.025		28°57.0N	130°12.0E	USING BALLPORE TREATMENT SYSTEM
5/27/10	DEBALLAST	41,315 KAS	43.2 KAS		1.025	OCEAN	32°38.2N	133°04.4E	USING BALLPORE TREATMENT SYSTEM
		41,416	158						
6/01/10	BALLAST	33,834	129.1	OCEAN	1.025		30-35.0N	139-14.0E	USING BALLPORE TREATMENT SYSTEM
6/4/10	DEBALLAST	33,834	129.1		1.025	OCEAN	16-57.5N	143-38.7E	USING BALLPORE TREATMENT SYSTEM
5/3/12	BALLAST	65525	250	LONG BEACH / ABRA	1.025		PIER 0-54 LONG BEACH		STABILITY & TRIM
6/11/12	DISCH	32762	125			DEEP SEA	11°35'N	088°31W	ARRIVAL DRAFTS EL SALADOR
6/19/12	DISCH	32762	125			DEEP SEA	19°16'N	105°15W	RINSE & SPIT
11/19/12	UPTAKE	45868	175	DEEP SEA	1.025		19°30'N	105°34W	TREER VIA TROJAN UV TREAT. SYS
11/23/12	DISCH	20968	80			DEEP SEA	33°55'N	116°35W	TRIM / STABILITY
11/26/12	DISCH	24900	95			LA CHANNEL	Port of LA BERTH 91		UV TREATED - TRIM & STABILITY
7/24/12	BALLAST	89018	340	Carg Strts.	1.025		CMA PIER		BULK SOLID WATER
7/25/12	TRANSFER	56875	217			3-154-1	CMA PIER		TROJAN TESTING
8/14/12	UPTAKE	48630	300	CARG. STRTS.	1.025		CMA PIER		TROJAN TESTING
8/17/12	TRANSFER	54516.8	208		1.010	3-154-1	CMA PIER		TROJAN TESTING
8/23/12	BALLAST	78630	300	CARG. STRTS	1.010		CMA PIER		TROJAN TESTING
8/30/12	TRANSFER	78630	300		1.010	3-154-1 / 5-104-1	CMA PIER		TROJAN TESTING
10/07/12	BALLAST	45867	175	SE BAY	1.010	SE BAY			COUNTER BALLAST
10/31/12	BALLAST	89114	340	CARG. STRTS	1.010	3-154-1 / 5-104-1	CMA PIER		TROJAN TESTING

TANK ACTIVITY RECORD

TANK NAME: 3-174-2

TYPE: SWB

HEIGHT: 26' 02"

CAPACITY: 96341 gal / 368 LT (SW)

DATE	ACTION	AMOUNT		FILL SOURCE	SPECIFIC GRAVITY	DISCHARGE TO	POSITION		REMARKS
		Gallons	Tons				Lat.	Long.	
5/2/12	BALLAST	15726	60	OCEAN	1.025		34.31.1	121-02.2	LIST CONTROL
5/3/12	BALLAST	65525	250	LONG BEACH HAR.	1.025		PIER D-54	LONG BEACH	STABILITY TRIM
6/11/12	DISCH	32762	125			DEEP SEA	11°35'N	088°31'W	ARRIVAL DRAFTS EL SALVADOR
6/19/12	DISCH	32762	125			DEEP SEA	19°16'N	105°15'W	RINSE & SPIT
6/19/12	UPTAKE	45868	175	DEEP SEA	1.025		19°30'N	105°34'W	TREATED VIA TROTAN UV TREAT. SH
6/23/12	DISCH	20968	80			DEEP SEA	33°55'N	116°35'W	TRIM / STABILITY
6/24/12	DISCH	24900	95			LA CHANNEL	PORT OF LA	BERTH 91	UV TREATED / TRIM & STABILITY
7/2/12	BALLAST	91636	350	CARGO STRS	1.025		CMA	PIER	BUTTS SOURCE WATER
7/25/12	TRANSFER	57602	220			3-154-2	CMA	PIER	TROSAN TESTING
8/14/12	UPTAKE	77635	297	CARGO STRS	1.025		CMA	PIER	TROSAN TESTING
8/17/12	TRANSFER	54517	208		1.010	3-154-2	CMA	PIER	TROSAN TESTING
8/23/12	BALLAST	78630	300	CARGO STRS	1.010		CMA	PIER	TROSAN TESTING
8/30/12	TRANSFER	78630	300		1.010	3-154-2 / 5-104-2	CMA	PIER	TROSAN TESTING
10/17/12	BALLAST	89114	340	CARGO STRS	1.010	3-154-2 / 5-104-2	CMA	PIER	TROSAN TESTING
5/10/13	DISCH.	89114	340	CARGO STRS	1.008	CARGO STRS	CMA	PIER	TROSAN TESTING

Appendix E GBF Automation System Outputs

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
1	17-Mar-12	12:25	3.8	0.0	18.1	Finished
1	17-Mar-12	12:26	209.1	0.0	13.8	Sea-to-Sea
1	17-Mar-12	12:27	187.8	0.0	13.2	Sea-to-Sea
1	17-Mar-12	12:28	194.6	0.0	12.8	Sea-to-Sea
1	17-Mar-12	12:29	201.6	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:30	203.6	0.0	13.1	Sea-to-Sea
1	17-Mar-12	12:31	207.0	0.0	12.8	Sea-to-Sea
1	17-Mar-12	12:32	244.9	0.0	13.0	Sea-to-Sea
1	17-Mar-12	12:33	245.3	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:34	225.2	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:35	204.4	0.0	13.1	Sea-to-Sea
1	17-Mar-12	12:36	202.3	0.0	12.7	Sea-to-Sea
1	17-Mar-12	12:37	201.4	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:38	201.5	0.0	13.1	Sea-to-Sea
1	17-Mar-12	12:39	212.1	0.0	13.1	Sea-to-Sea
1	17-Mar-12	12:40	244.0	0.0	12.8	Sea-to-Sea
1	17-Mar-12	12:41	242.8	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:42	243.5	0.0	13.1	Sea-to-Sea
1	17-Mar-12	12:43	246.1	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:44	228.2	0.0	13.2	Sea-to-Sea
1	17-Mar-12	12:45	190.4	0.0	13.1	Sea-to-Sea
1	17-Mar-12	12:46	196.9	0.0	12.7	Sea-to-Sea
1	17-Mar-12	12:47	194.7	0.0	13.1	Sea-to-Sea
1	17-Mar-12	12:48	196.0	0.0	13.2	Sea-to-Sea
1	17-Mar-12	12:49	197.6	0.0	12.7	Sea-to-Sea
1	17-Mar-12	12:50	196.3	0.0	12.7	Sea-to-Sea
1	17-Mar-12	12:51	203.3	0.0	13.1	Sea-to-Sea
1	17-Mar-12	12:52	245.3	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:53	244.4	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:54	244.9	0.0	12.9	Sea-to-Sea
1	17-Mar-12	12:55	244.9	0.0	12.8	Sea-to-Sea
1	17-Mar-12	12:56	245.7	0.0	12.8	Sea-to-Sea
1	17-Mar-12	12:57	244.0	0.0	13.0	Sea-to-Sea
1	17-Mar-12	12:58	222.2	0.0	12.8	Sea-to-Sea
1	17-Mar-12	12:59	195.5	0.0	13.1	Sea-to-Sea
1	17-Mar-12	13:00	194.2	0.0	12.7	Sea-to-Sea
1	17-Mar-12	13:01	195.9	0.0	13.0	Sea-to-Sea
1	17-Mar-12	13:02	198.0	0.0	12.9	Sea-to-Sea
1	17-Mar-12	13:03	198.5	0.0	12.7	Sea-to-Sea
1	17-Mar-12	13:04	235.1	0.0	12.9	Sea-to-Sea
1	17-Mar-12	13:05	244.8	0.0	13.0	Ready to Sample
1	17-Mar-12	13:06	250.4	0.0	13.0	Ready to Sample
1	17-Mar-12	13:07	244.9	3.2	13.1	Treatment Uptake
1	17-Mar-12	13:08	241.5	6.7	13.2	Treatment Uptake
1	17-Mar-12	13:09	199.6	10.3	13.1	Treatment Uptake
1	17-Mar-12	13:10	199.6	14.0	12.9	Treatment Uptake
1	17-Mar-12	13:11	200.7	17.7	13.2	Treatment Uptake
1	17-Mar-12	13:12	202.1	21.3	12.8	Treatment Uptake
1	17-Mar-12	13:13	200.1	24.8	13.2	Treatment Uptake
1	17-Mar-12	13:14	195.4	28.4	12.8	Treatment Uptake
1	17-Mar-12	13:15	188.8	31.8	12.8	Treatment Uptake
1	17-Mar-12	13:16	192.1	35.3	13.2	Treatment Uptake
1	17-Mar-12	13:17	221.4	38.8	13.2	Treatment Uptake
1	17-Mar-12	13:18	226.6	42.2	12.9	Treatment Uptake

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
1	17-Mar-12	13:19	247.4	45.6	12.9	Treatment Uptake
1	17-Mar-12	13:20	249.0	49.2	13.2	Treatment Uptake
1	17-Mar-12	13:21	248.7	52.7	13.2	Treatment Uptake
1	17-Mar-12	13:22	248.7	56.2	13.2	Treatment Uptake
1	17-Mar-12	13:23	251.0	59.7	13.1	Treatment Uptake
1	17-Mar-12	13:24	190.2	63.2	13.1	Treatment Uptake
1	17-Mar-12	13:25	190.4	66.9	12.9	Treatment Uptake
1	17-Mar-12	13:26	193.1	70.4	13.0	Treatment Uptake
1	17-Mar-12	13:27	194.2	73.9	13.1	Treatment Uptake
1	17-Mar-12	13:28	193.8	77.4	12.9	Treatment Uptake
1	17-Mar-12	13:29	197.7	81.0	13.0	Treatment Uptake
1	17-Mar-12	13:30	200.3	84.6	13.1	Treatment Uptake
1	17-Mar-12	13:31	200.3	88.2	13.2	Treatment Uptake
1	17-Mar-12	13:32	201.0	91.7	12.9	Treatment Uptake
1	17-Mar-12	13:33	199.3	95.2	13.2	Treatment Uptake
1	17-Mar-12	13:34	199.7	98.7	12.7	Treatment Uptake
1	17-Mar-12	13:35	203.2	102.2	13.2	Treatment Uptake
1	17-Mar-12	13:36	225.3	105.7	13.1	Treatment Uptake
1	17-Mar-12	13:37	245.3	109.2	13.2	Treatment Uptake
1	17-Mar-12	13:38	244.4	112.7	12.8	Treatment Uptake
1	17-Mar-12	13:39	246.9	116.2	13.1	Treatment Uptake
1	17-Mar-12	13:40	246.9	119.7	13.1	Treatment Uptake
1	17-Mar-12	13:41	248.7	123.3	12.9	Treatment Uptake
1	17-Mar-12	13:42	230.6	126.9	13.0	Treatment Uptake
1	17-Mar-12	13:43	207.4	130.5	13.2	Treatment Uptake
1	17-Mar-12	13:44	241.9	134.0	13.3	Treatment Uptake
1	17-Mar-12	13:45	193.3	137.3	12.9	Treatment Uptake
1	17-Mar-12	13:46	211.5	140.9	13.1	Treatment Uptake
1	17-Mar-12	13:47	242.3	144.3	12.9	Treatment Uptake
1	17-Mar-12	13:48	195.9	147.7	13.2	Treatment Uptake
1	17-Mar-12	13:49	212.5	151.2	13.2	Treatment Uptake
1	17-Mar-12	13:50	242.3	154.5	13.4	Treatment Uptake
1	17-Mar-12	13:51	189.1	157.9	13.0	Treatment Uptake
1	17-Mar-12	13:52	212.9	161.4	13.2	Treatment Uptake
1	17-Mar-12	13:53	249.1	164.7	12.9	Treatment Uptake
1	17-Mar-12	13:54	201.8	168.3	13.0	Treatment Uptake
1	17-Mar-12	13:55	218.0	171.7	13.1	Treatment Uptake
1	17-Mar-12	13:56	172.2	174.9	12.9	Treatment Uptake
1	17-Mar-12	13:57	214.6	178.1	13.3	Treatment Uptake
1	17-Mar-12	13:58	241.6	181.2	13.1	Treatment Uptake
1	17-Mar-12	13:59	189.1	184.7	12.9	Treatment Uptake
1	17-Mar-12	14:00	215.1	188.1	12.9	Treatment Uptake
1	17-Mar-12	14:01	233.4	191.2	13.1	Treatment Uptake
1	17-Mar-12	14:02	197.6	194.9	12.9	Treatment Uptake
1	17-Mar-12	14:03	218.3	198.3	13.0	Treatment Uptake
1	17-Mar-12	14:04	212.0	201.5	12.9	Treatment Uptake
1	17-Mar-12	14:05	191.2	205.1	13.0	Treatment Uptake
1	17-Mar-12	14:06	189.0	208.4	13.3	Treatment Uptake
1	17-Mar-12	14:07	230.4	211.2	12.9	Control Uptake
1	17-Mar-12	14:08	249.1	215.3	13.1	Control Uptake
1	17-Mar-12	14:09	248.7	219.4	13.1	Control Uptake
1	17-Mar-12	14:10	251.2	223.5	13.0	Control Uptake
1	17-Mar-12	14:11	252.2	227.6	12.7	Control Uptake
1	17-Mar-12	14:12	250.8	231.7	12.8	Control Uptake

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
1	17-Mar-12	14:13	252.1	235.7	12.7	Control Uptake
1	17-Mar-12	14:14	251.7	239.9	13.2	Control Uptake
1	17-Mar-12	14:15	252.5	243.9	12.7	Control Uptake
1	17-Mar-12	14:16	250.8	248.0	13.1	Control Uptake
1	17-Mar-12	14:17	251.2	252.1	12.6	Control Uptake
1	17-Mar-12	14:18	251.8	256.2	12.7	Control Uptake
1	17-Mar-12	14:19	252.0	260.3	12.7	Control Uptake
1	17-Mar-12	14:20	252.5	264.4	12.7	Control Uptake
1	17-Mar-12	14:21	252.1	268.5	12.8	Control Uptake
1	17-Mar-12	14:22	252.2	272.6	12.5	Control Uptake
1	17-Mar-12	14:23	252.0	276.7	13.0	Control Uptake
1	17-Mar-12	14:24	252.0	280.7	12.4	Control Uptake
1	17-Mar-12	14:25	252.9	284.8	12.9	Control Uptake
1	17-Mar-12	14:26	253.4	288.9	12.3	Control Uptake
1	17-Mar-12	14:27	250.4	293.0	12.5	Control Uptake
1	17-Mar-12	14:28	253.8	297.1	12.7	Control Uptake
1	17-Mar-12	14:29	250.9	301.2	12.5	Control Uptake
1	17-Mar-12	14:30	252.5	305.3	12.5	Control Uptake
1	17-Mar-12	14:31	251.2	309.4	12.5	Control Uptake
1	17-Mar-12	14:32	251.8	313.4	12.5	Control Uptake
1	17-Mar-12	14:33	253.3	317.5	12.9	Control Uptake
1	17-Mar-12	14:34	253.0	321.6	13.0	Control Uptake
1	17-Mar-12	14:35	252.1	325.7	13.2	Control Uptake
1	17-Mar-12	14:36	252.1	329.9	13.1	Control Uptake
1	17-Mar-12	14:37	252.5	333.9	12.7	Control Uptake
1	17-Mar-12	14:38	252.1	338.0	12.9	Control Uptake
1	17-Mar-12	14:39	251.5	342.1	12.8	Control Uptake
1	17-Mar-12	14:40	252.1	346.2	12.7	Control Uptake
1	17-Mar-12	14:41	252.5	350.3	12.8	Control Uptake
1	17-Mar-12	14:42	252.0	354.4	13.2	Control Uptake
1	17-Mar-12	14:43	252.1	358.4	13.4	Control Uptake
1	17-Mar-12	14:44	252.5	362.5	12.9	Control Uptake
1	17-Mar-12	14:45	251.2	366.6	13.0	Control Uptake
1	17-Mar-12	14:46	252.2	370.8	13.3	Control Uptake
1	17-Mar-12	14:47	252.1	374.8	13.4	Control Uptake
1	17-Mar-12	14:48	252.1	378.9	13.0	Control Uptake
1	17-Mar-12	14:49	252.1	382.9	13.2	Control Uptake
1	17-Mar-12	14:50	252.4	387.0	13.3	Control Uptake
1	17-Mar-12	14:51	251.7	391.1	13.4	Control Uptake
1	17-Mar-12	14:52	251.2	395.3	12.9	Control Uptake
1	17-Mar-12	14:53	251.7	399.4	13.2	Control Uptake
1	17-Mar-12	14:54	252.2	403.5	13.2	Control Uptake
1	17-Mar-12	14:55	251.3	407.5	13.1	Control Uptake
1	17-Mar-12	14:56	252.0	411.6	13.2	Control Uptake
1	17-Mar-12	14:57	251.2	415.7	13.5	Control Uptake
1	17-Mar-12	14:58	0.0	416.5	13.1	Finished
1	22-Mar-12	11:45	0.0	0.0	14.7	Finished
1	22-Mar-12	11:46	233.8	0.0	20.5	Recirculation
1	22-Mar-12	11:47	241.0	0.0	19.5	Recirculation
1	22-Mar-12	11:48	235.5	0.0	19.3	Recirculation
1	22-Mar-12	11:49	234.6	0.0	20.1	Recirculation
1	22-Mar-12	11:50	146.9	0.0	19.7	Recirculation
1	22-Mar-12	11:51	257.2	0.0	16.4	Recirculation
1	22-Mar-12	11:52	252.7	0.0	14.8	Recirculation

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
1	22-Mar-12	11:53	244.5	0.0	15.1	Recirculation
1	22-Mar-12	11:54	243.5	0.0	15.6	Recirculation
1	22-Mar-12	11:55	244.0	0.0	15.5	Recirculation
1	22-Mar-12	11:56	246.2	0.0	15.4	Recirculation
1	22-Mar-12	11:57	241.0	0.0	15.8	Recirculation
1	22-Mar-12	11:58	239.0	0.0	15.6	Recirculation
1	22-Mar-12	11:59	239.8	0.0	16.0	Recirculation
1	22-Mar-12	12:00	239.0	0.0	15.7	Recirculation
1	22-Mar-12	12:01	241.9	0.0	15.9	Recirculation
1	22-Mar-12	12:02	240.1	0.0	16.3	Recirculation
1	22-Mar-12	12:03	241.9	0.0	16.0	Recirculation
1	22-Mar-12	12:04	238.5	0.0	16.3	Recirculation
1	22-Mar-12	12:05	241.3	0.0	16.2	Recirculation
1	22-Mar-12	12:06	239.3	0.0	16.3	Recirculation
1	22-Mar-12	12:07	238.9	0.0	16.5	Recirculation
1	22-Mar-12	12:08	239.8	0.0	16.1	Recirculation
1	22-Mar-12	12:09	239.8	0.0	16.3	Recirculation
1	22-Mar-12	12:10	240.4	0.0	16.6	Recirculation
1	22-Mar-12	12:11	239.9	0.0	16.6	Recirculation
1	22-Mar-12	12:12	239.5	0.0	16.2	Recirculation
1	22-Mar-12	12:13	239.8	0.0	16.7	Recirculation
1	22-Mar-12	12:14	241.5	0.0	16.3	Recirculation
1	22-Mar-12	12:15	239.3	0.0	16.6	Recirculation
1	22-Mar-12	12:16	240.2	2.1	16.6	Treatment Discharge
1	22-Mar-12	12:17	241.2	6.2	14.4	Treatment Discharge
1	22-Mar-12	12:18	240.2	10.3	13.9	Treatment Discharge
1	22-Mar-12	12:19	240.2	14.4	13.8	Treatment Discharge
1	22-Mar-12	12:20	240.8	18.5	14.3	Treatment Discharge
1	22-Mar-12	12:21	240.2	22.6	13.9	Treatment Discharge
1	22-Mar-12	12:22	240.1	26.7	14.3	Treatment Discharge
1	22-Mar-12	12:23	240.0	30.7	14.0	Treatment Discharge
1	22-Mar-12	12:24	241.5	34.8	13.8	Treatment Discharge
1	22-Mar-12	12:25	240.6	38.9	14.2	Treatment Discharge
1	22-Mar-12	12:26	240.2	43.1	13.8	Treatment Discharge
1	22-Mar-12	12:27	240.5	47.2	14.3	Treatment Discharge
1	22-Mar-12	12:28	240.5	51.2	14.1	Treatment Discharge
1	22-Mar-12	12:29	240.8	55.3	14.2	Treatment Discharge
1	22-Mar-12	12:30	241.8	59.4	14.0	Treatment Discharge
1	22-Mar-12	12:31	241.0	63.5	13.9	Treatment Discharge
1	22-Mar-12	12:32	239.8	67.6	14.2	Treatment Discharge
1	22-Mar-12	12:33	240.2	71.7	14.1	Treatment Discharge
1	22-Mar-12	12:34	241.0	75.7	14.1	Treatment Discharge
1	22-Mar-12	12:35	240.2	79.8	14.1	Treatment Discharge
1	22-Mar-12	12:36	241.0	83.9	14.1	Treatment Discharge
1	22-Mar-12	12:37	240.2	88.0	14.1	Treatment Discharge
1	22-Mar-12	12:38	241.5	92.1	13.9	Treatment Discharge
1	22-Mar-12	12:39	240.2	96.2	13.8	Treatment Discharge
1	22-Mar-12	12:40	240.5	100.2	13.9	Treatment Discharge
1	22-Mar-12	12:41	240.6	104.3	13.8	Treatment Discharge
1	22-Mar-12	12:42	241.0	108.5	13.8	Treatment Discharge
1	22-Mar-12	12:43	241.0	112.6	14.2	Treatment Discharge
1	22-Mar-12	12:44	240.2	116.7	14.0	Treatment Discharge
1	22-Mar-12	12:45	241.2	120.8	13.8	Treatment Discharge
1	22-Mar-12	12:46	240.2	124.8	14.2	Treatment Discharge
1	22-Mar-12	12:47	241.5	128.9	13.9	Treatment Discharge

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
1	22-Mar-12	12:48	239.8	133.0	14.3	Treatment Discharge
1	22-Mar-12	12:49	240.2	137.1	13.9	Treatment Discharge
1	22-Mar-12	12:50	241.5	141.2	13.9	Treatment Discharge
1	22-Mar-12	12:51	240.3	145.3	14.2	Treatment Discharge
1	22-Mar-12	12:52	239.5	149.3	13.8	Treatment Discharge
1	22-Mar-12	12:53	241.2	153.5	13.8	Treatment Discharge
1	22-Mar-12	12:54	240.5	157.5	13.7	Treatment Discharge
1	22-Mar-12	12:55	240.2	161.6	13.9	Treatment Discharge
1	22-Mar-12	12:56	241.4	165.7	14.3	Treatment Discharge
1	22-Mar-12	12:57	241.3	169.8	13.8	Treatment Discharge
1	22-Mar-12	12:58	120.9	173.4	14.0	Treatment Discharge / Stripping
1	22-Mar-12	12:59	47.3	174.6	14.3	Treatment Discharge / Stripping
1	22-Mar-12	13:00	47.7	175.3	14.3	Treatment Discharge / Stripping
1	22-Mar-12	13:01	47.6	176.2	14.3	Treatment Discharge / Stripping
1	22-Mar-12	13:02	47.4	177.0	13.9	Treatment Discharge / Stripping
1	22-Mar-12	13:03	47.3	177.8	14.4	Treatment Discharge / Stripping
1	22-Mar-12	13:04	47.3	178.5	14.4	Treatment Discharge / Stripping
1	22-Mar-12	13:05	47.7	179.3	13.9	Treatment Discharge / Stripping
1	22-Mar-12	13:06	47.3	180.1	14.3	Treatment Discharge / Stripping
1	22-Mar-12	13:07	47.3	180.8	14.2	Treatment Discharge / Stripping
1	22-Mar-12	13:08	46.8	181.5	14.2	Treatment Discharge / Stripping
1	22-Mar-12	13:09	0.0	182.1	14.3	Finished
1	22-Mar-12	14:40	251.7	0.0	14.4	Finished
1	22-Mar-12	14:41	256.8	0.0	14.6	Recirculation
1	22-Mar-12	14:42	262.3	0.0	14.7	Recirculation
1	22-Mar-12	14:43	252.1	0.0	14.9	Recirculation
1	22-Mar-12	14:44	252.4	0.0	15.1	Recirculation
1	22-Mar-12	14:45	253.8	0.0	15.0	Recirculation
1	22-Mar-12	14:46	251.0	0.0	15.2	Recirculation
1	22-Mar-12	14:47	253.8	0.0	15.6	Recirculation
1	22-Mar-12	14:48	254.5	0.0	15.8	Recirculation
1	22-Mar-12	14:49	250.8	0.0	15.8	Recirculation
1	22-Mar-12	14:50	253.8	0.0	15.9	Recirculation
1	22-Mar-12	14:51	252.9	0.0	15.8	Recirculation
1	22-Mar-12	14:52	253.9	0.0	15.7	Recirculation
1	22-Mar-12	14:53	253.4	0.0	16.0	Recirculation
1	22-Mar-12	14:54	252.7	0.0	16.5	Recirculation
1	22-Mar-12	14:55	252.9	0.0	16.3	Recirculation
1	22-Mar-12	14:56	252.5	0.0	16.6	Recirculation
1	22-Mar-12	14:57	252.4	0.0	16.2	Recirculation
1	22-Mar-12	14:58	253.8	0.0	16.5	Recirculation
1	22-Mar-12	14:59	252.5	0.0	16.3	Recirculation
1	22-Mar-12	15:00	252.5	0.0	16.8	Recirculation
1	22-Mar-12	15:01	253.5	0.0	17.0	Recirculation
1	22-Mar-12	15:02	251.7	0.0	16.5	Recirculation
1	22-Mar-12	15:03	253.4	0.0	16.8	Recirculation
1	22-Mar-12	15:04	252.5	0.0	16.6	Recirculation
1	22-Mar-12	15:05	253.3	0.0	17.0	Recirculation
1	22-Mar-12	15:06	252.8	0.0	17.4	Ready to Sample
1	22-Mar-12	15:07	249.5	3.3	14.8	Control Discharge
1	22-Mar-12	15:08	249.4	7.4	14.3	Control Discharge
1	22-Mar-12	15:09	249.1	11.5	14.1	Control Discharge
1	22-Mar-12	15:10	249.1	15.6	14.3	Control Discharge
1	22-Mar-12	15:11	249.1	19.7	13.9	Control Discharge

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
1	22-Mar-12	15:12	250.0	23.7	13.8	Control Discharge
1	22-Mar-12	15:13	249.0	27.8	14.0	Control Discharge
1	22-Mar-12	15:14	249.5	31.9	14.3	Control Discharge
1	22-Mar-12	15:15	249.2	36.0	14.3	Control Discharge
1	22-Mar-12	15:16	249.1	40.2	14.2	Control Discharge
1	22-Mar-12	15:17	249.3	44.2	13.8	Control Discharge
1	22-Mar-12	15:18	249.5	48.3	14.2	Control Discharge
1	22-Mar-12	15:19	250.0	52.4	13.9	Control Discharge
1	22-Mar-12	15:20	250.0	56.5	14.2	Control Discharge
1	22-Mar-12	15:21	249.2	60.5	13.8	Control Discharge
1	22-Mar-12	15:22	249.1	64.7	14.2	Control Discharge
1	22-Mar-12	15:23	249.1	68.7	13.8	Control Discharge
1	22-Mar-12	15:24	249.1	72.8	13.9	Control Discharge
1	22-Mar-12	15:25	249.6	76.9	13.8	Control Discharge
1	22-Mar-12	15:26	249.1	81.0	14.2	Control Discharge
1	22-Mar-12	15:27	249.1	85.1	13.8	Control Discharge
1	22-Mar-12	15:28	249.2	89.2	13.8	Control Discharge
1	22-Mar-12	15:29	249.5	93.2	14.1	Control Discharge
1	22-Mar-12	15:30	249.6	97.3	13.9	Control Discharge
1	22-Mar-12	15:31	249.1	101.4	13.9	Control Discharge
1	22-Mar-12	15:32	249.0	105.5	14.0	Control Discharge
1	22-Mar-12	15:33	249.1	109.7	14.2	Control Discharge
1	22-Mar-12	15:34	250.1	113.8	14.3	Control Discharge
1	22-Mar-12	15:35	249.5	117.8	14.1	Control Discharge
1	22-Mar-12	15:36	249.1	121.9	14.1	Control Discharge
1	22-Mar-12	15:37	249.9	126.0	14.4	Control Discharge
1	22-Mar-12	15:38	249.0	130.1	14.1	Control Discharge
1	22-Mar-12	15:39	249.1	134.2	13.9	Control Discharge
1	22-Mar-12	15:40	250.4	138.3	14.1	Control Discharge
1	22-Mar-12	15:41	249.1	142.3	13.8	Control Discharge
1	22-Mar-12	15:42	249.4	146.4	14.2	Control Discharge
1	22-Mar-12	15:43	249.1	150.5	14.0	Control Discharge
1	22-Mar-12	15:44	248.7	154.6	14.0	Control Discharge
1	22-Mar-12	15:45	249.4	158.7	14.2	Control Discharge
1	22-Mar-12	15:46	249.1	162.8	14.3	Control Discharge
1	22-Mar-12	15:47	248.7	166.8	13.9	Control Discharge
1	22-Mar-12	15:48	250.1	170.9	14.2	Control Discharge
1	22-Mar-12	15:49	249.5	175.1	14.0	Control Discharge
1	22-Mar-12	15:50	249.9	179.2	14.2	Control Discharge
1	22-Mar-12	15:51	143.4	183.0	14.3	Control Discharge / Stripping
1	22-Mar-12	15:52	47.3	183.9	14.3	Control Discharge / Stripping
1	22-Mar-12	15:53	49.7	184.7	14.3	Control Discharge / Stripping
1	22-Mar-12	15:54	49.8	185.6	14.2	Control Discharge / Stripping
1	22-Mar-12	15:55	49.7	186.4	14.0	Control Discharge / Stripping
1	22-Mar-12	15:56	49.8	187.3	14.3	Control Discharge / Stripping
1	22-Mar-12	15:57	49.5	188.1	14.0	Control Discharge / Stripping
1	22-Mar-12	15:58	49.4	189.0	14.1	Control Discharge / Stripping
1	22-Mar-12	15:59	49.1	189.8	14.4	Control Discharge / Stripping
1	22-Mar-12	16:00	49.4	190.7	14.0	Control Discharge / Stripping
1	22-Mar-12	16:01	49.5	191.5	13.8	Control Discharge / Stripping
1	22-Mar-12	16:02	49.4	192.3	13.9	Control Discharge / Stripping
1	22-Mar-12	16:03	49.4	193.1	14.2	Control Discharge / Stripping
1	22-Mar-12	16:04	49.0	193.9	14.4	Control Discharge / Stripping
1	22-Mar-12	16:05	20.9	194.6	14.3	Control Discharge / Stripping

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
1	22-Mar-12	16:06	0.0	194.6	14.1	Finished
2	1-May-12	17:20	0.0	0.0	24.9	Finished
2	1-May-12	17:21	213.4	0.0	23.8	Recirculation
2	1-May-12	17:23	250.8	0.0	16.0	Recirculation
2	1-May-12	17:24	250.9	0.0	15.8	Recirculation
2	1-May-12	17:25	253.0	0.0	16.0	Recirculation
2	1-May-12	17:26	210.4	0.0	15.7	Recirculation
2	1-May-12	17:27	253.7	0.0	16.2	Recirculation
2	1-May-12	17:28	215.1	0.0	16.2	Sea-to-Sea
2	1-May-12	17:29	253.3	0.0	16.2	Sea-to-Sea
2	1-May-12	17:30	230.4	0.0	16.1	Sea-to-Sea
2	1-May-12	17:31	252.5	0.0	16.2	Sea-to-Sea
2	1-May-12	17:32	253.7	0.0	16.2	Sea-to-Sea
2	1-May-12	17:33	252.8	0.0	16.2	Sea-to-Sea
2	1-May-12	17:34	255.5	0.0	15.8	Sea-to-Sea
2	1-May-12	17:35	254.7	0.0	16.2	Sea-to-Sea
2	1-May-12	17:36	206.1	0.0	16.2	Sea-to-Sea
2	1-May-12	17:37	214.2	0.0	16.0	Sea-to-Sea
2	1-May-12	17:38	253.4	0.0	16.2	Sea-to-Sea
2	1-May-12	17:39	214.2	0.0	14.8	Sea-to-Sea
2	1-May-12	17:40	259.9	0.0	15.0	Ready to Sample
2	1-May-12	17:41	241.5	3.1	14.9	Treatment Uptake
2	1-May-12	17:42	253.8	7.2	15.3	Treatment Uptake
2	1-May-12	17:43	242.9	11.3	15.3	Treatment Uptake
2	1-May-12	17:44	252.5	15.1	14.5	Treatment Uptake
2	1-May-12	17:45	253.3	19.2	14.8	Treatment Uptake
2	1-May-12	17:46	184.3	22.7	14.4	Treatment Uptake
2	1-May-12	17:47	252.5	26.7	14.8	Treatment Uptake
2	1-May-12	17:48	253.0	30.8	14.4	Treatment Uptake
2	1-May-12	17:49	253.8	34.4	14.9	Treatment Uptake
2	1-May-12	17:50	253.8	38.5	14.8	Treatment Uptake
2	1-May-12	17:51	200.4	42.2	14.4	Treatment Uptake
2	1-May-12	17:52	253.8	46.1	14.5	Treatment Uptake
2	1-May-12	17:54	209.9	50.1	14.0	Treatment Uptake
2	1-May-12	17:55	253.5	53.9	14.6	Treatment Uptake
2	1-May-12	17:56	252.5	58.0	14.6	Treatment Uptake
2	1-May-12	17:57	253.4	61.6	14.1	Treatment Uptake
2	1-May-12	17:58	252.4	65.7	14.5	Treatment Uptake
2	1-May-12	17:59	210.3	69.5	14.4	Treatment Uptake
2	1-May-12	18:00	253.0	73.4	14.8	Treatment Uptake
2	1-May-12	18:01	214.5	77.5	14.6	Treatment Uptake
2	1-May-12	18:02	253.4	81.3	14.3	Treatment Uptake
2	1-May-12	18:03	226.1	85.3	14.7	Treatment Uptake
2	1-May-12	18:04	253.4	89.0	13.5	Treatment Uptake
2	1-May-12	18:05	218.5	93.0	13.0	Treatment Uptake
2	1-May-12	18:06	253.4	96.7	13.0	Treatment Uptake
2	1-May-12	18:07	252.4	100.8	13.4	Treatment Uptake
2	1-May-12	18:08	254.4	104.5	13.6	Treatment Uptake
2	1-May-12	18:09	253.8	108.6	13.0	Treatment Uptake
2	1-May-12	18:10	218.5	112.3	13.5	Treatment Uptake
2	1-May-12	18:11	252.5	116.4	13.2	Treatment Uptake
2	1-May-12	18:12	221.4	120.4	13.2	Treatment Uptake
2	1-May-12	18:13	253.3	124.4	13.2	Treatment Uptake
2	1-May-12	18:14	252.5	128.4	13.2	Treatment Uptake

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
2	1-May-12	18:15	253.3	132.3	13.1	Treatment Uptake
2	1-May-12	18:16	252.5	136.3	13.3	Treatment Uptake
2	1-May-12	18:17	209.5	140.1	13.8	Treatment Uptake
2	1-May-12	18:18	253.4	144.2	13.8	Treatment Uptake
2	1-May-12	18:19	230.0	148.2	13.7	Treatment Uptake
2	1-May-12	18:20	252.8	152.1	13.4	Treatment Uptake
2	1-May-12	18:21	252.6	156.2	13.5	Treatment Uptake
2	1-May-12	18:22	253.1	160.0	13.4	Treatment Uptake
2	1-May-12	18:23	253.5	164.1	13.2	Treatment Uptake
2	1-May-12	18:24	215.2	168.0	13.1	Treatment Uptake
2	1-May-12	18:25	253.8	172.0	13.3	Treatment Uptake
2	1-May-12	18:26	232.1	176.1	13.2	Treatment Uptake
2	1-May-12	18:27	253.4	179.9	13.4	Treatment Uptake
2	1-May-12	18:28	253.4	184.0	13.1	Treatment Uptake
2	1-May-12	18:29	265.4	187.8	13.3	Treatment Uptake
2	1-May-12	18:30	253.1	191.9	12.1	Treatment Uptake
2	1-May-12	18:31	229.5	196.0	12.1	Treatment Uptake
2	1-May-12	18:32	252.8	199.8	12.0	Treatment Uptake
2	1-May-12	18:33	253.2	203.7	12.0	Finished
2	1-May-12	18:34	251.1	204.1	11.5	Control Uptake
2	1-May-12	18:35	243.0	208.2	11.6	Control Uptake
2	1-May-12	18:36	252.1	212.3	12.3	Control Uptake
2	1-May-12	18:37	253.0	216.3	12.5	Control Uptake
2	1-May-12	18:38	252.6	220.4	12.1	Control Uptake
2	1-May-12	18:39	253.4	224.6	11.8	Control Uptake
2	1-May-12	18:40	253.8	228.6	11.1	Control Uptake
2	1-May-12	18:41	254.5	232.7	10.8	Control Uptake
2	1-May-12	18:42	252.5	236.8	10.8	Control Uptake
2	1-May-12	18:43	253.4	240.9	10.6	Control Uptake
2	1-May-12	18:44	253.0	245.0	10.5	Control Uptake
2	1-May-12	18:45	254.2	249.1	10.3	Control Uptake
2	1-May-12	18:46	253.3	253.1	10.5	Control Uptake
2	1-May-12	18:47	253.0	257.2	10.4	Control Uptake
2	1-May-12	18:48	253.1	261.3	10.3	Control Uptake
2	1-May-12	18:49	254.3	265.4	10.9	Control Uptake
2	1-May-12	18:50	252.1	269.6	10.9	Control Uptake
2	1-May-12	18:51	253.4	273.6	11.0	Control Uptake
2	1-May-12	18:52	254.2	277.7	11.1	Control Uptake
2	1-May-12	18:53	253.5	281.8	11.2	Control Uptake
2	1-May-12	18:54	253.8	285.8	11.2	Control Uptake
2	1-May-12	18:55	253.3	289.9	11.0	Control Uptake
2	1-May-12	18:56	251.8	294.1	11.2	Control Uptake
2	1-May-12	18:57	252.5	298.2	11.0	Control Uptake
2	1-May-12	18:58	252.1	302.2	10.4	Control Uptake
2	1-May-12	18:59	253.0	306.3	10.6	Control Uptake
2	1-May-12	19:00	252.1	310.4	10.9	Control Uptake
2	1-May-12	19:01	252.1	314.5	10.7	Control Uptake
2	1-May-12	19:02	252.9	318.6	10.6	Control Uptake
2	1-May-12	19:03	252.5	322.7	10.9	Control Uptake
2	1-May-12	19:04	252.5	326.7	11.2	Control Uptake
2	1-May-12	19:05	253.2	330.8	10.7	Control Uptake
2	1-May-12	19:06	252.5	335.0	10.9	Control Uptake
2	1-May-12	19:07	253.0	339.1	11.1	Control Uptake
2	1-May-12	19:08	251.2	343.1	11.3	Control Uptake

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
2	1-May-12	19:09	253.8	347.2	11.1	Control Uptake
2	1-May-12	19:10	253.0	351.3	11.2	Control Uptake
2	1-May-12	19:11	253.4	355.3	11.1	Control Uptake
2	1-May-12	19:12	253.7	359.5	11.0	Control Uptake
2	1-May-12	19:13	252.1	363.6	11.5	Control Uptake
2	1-May-12	19:14	253.8	367.7	11.5	Control Uptake
2	1-May-12	19:15	252.6	371.8	11.5	Control Uptake
2	1-May-12	19:16	253.4	375.8	11.5	Control Uptake
2	1-May-12	19:17	252.5	379.9	11.1	Control Uptake
2	1-May-12	19:18	252.5	384.0	11.2	Control Uptake
2	1-May-12	19:19	252.5	388.1	11.2	Control Uptake
2	1-May-12	19:20	252.5	392.2	11.3	Control Uptake
2	1-May-12	19:21	252.5	396.3	11.5	Control Uptake
2	1-May-12	19:22	253.4	400.3	11.2	Control Uptake
2	1-May-12	19:23	253.2	404.5	11.5	Control Uptake
2	1-May-12	19:24	252.1	408.6	11.7	Control Uptake
2	1-May-12	19:25	253.8	412.6	11.3	Control Uptake
2	1-May-12	19:26	182.4	416.6	11.7	Control Uptake
2	1-May-12	19:27	0.0	416.8	11.5	Finished
2	6-May-12	13:33	0.0	0.0	24.4	Finished
2	6-May-12	13:34	236.6	0.0	23.4	Recirculation
2	6-May-12	13:35	227.0	0.0	23.5	Recirculation
2	6-May-12	13:36	247.0	0.0	22.9	Recirculation
2	6-May-12	13:37	245.7	0.0	19.4	Recirculation
2	6-May-12	13:38	243.9	0.0	19.9	Recirculation
2	6-May-12	13:39	244.9	0.0	19.9	Recirculation
2	6-May-12	13:40	243.7	0.0	20.3	Recirculation
2	6-May-12	13:41	243.6	0.0	20.3	Recirculation
2	6-May-12	13:42	244.4	0.0	20.3	Recirculation
2	6-May-12	13:43	244.9	0.0	20.5	Recirculation
2	6-May-12	13:44	245.2	0.0	20.7	Recirculation
2	6-May-12	13:45	244.0	1.2	20.9	Treatment Discharge
2	6-May-12	13:46	244.0	5.5	18.8	Treatment Discharge
2	6-May-12	13:47	242.7	9.8	18.6	Treatment Discharge
2	6-May-12	13:48	243.6	14.0	18.2	Treatment Discharge
2	6-May-12	13:49	244.3	18.2	18.5	Treatment Discharge
2	6-May-12	13:50	244.8	22.5	18.3	Treatment Discharge
2	6-May-12	13:51	243.2	26.7	18.1	Treatment Discharge
2	6-May-12	13:52	244.5	30.9	18.1	Treatment Discharge
2	6-May-12	13:53	244.9	35.3	18.4	Treatment Discharge
2	6-May-12	13:54	244.5	39.4	18.1	Treatment Discharge
2	6-May-12	13:55	244.9	43.7	18.4	Treatment Discharge
2	6-May-12	13:56	243.3	47.9	18.3	Treatment Discharge
2	6-May-12	13:57	243.5	52.2	18.1	Treatment Discharge
2	6-May-12	13:58	244.0	56.5	18.1	Treatment Discharge
2	6-May-12	13:59	244.8	60.8	18.0	Treatment Discharge
2	6-May-12	14:00	244.0	65.0	18.2	Treatment Discharge
2	6-May-12	14:01	244.4	69.2	18.4	Treatment Discharge
2	6-May-12	14:02	244.9	73.5	18.3	Treatment Discharge
2	6-May-12	14:03	245.3	77.7	18.3	Treatment Discharge
2	6-May-12	14:04	244.2	82.0	18.4	Treatment Discharge
2	6-May-12	14:05	245.3	86.3	18.4	Treatment Discharge
2	6-May-12	14:06	245.0	90.5	18.3	Treatment Discharge
2	6-May-12	14:07	245.3	94.8	18.0	Treatment Discharge

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
2	6-May-12	14:08	244.9	99.0	18.1	Treatment Discharge
2	6-May-12	14:09	244.4	103.3	18.4	Treatment Discharge
2	6-May-12	14:10	243.6	107.6	18.2	Treatment Discharge
2	6-May-12	14:11	244.9	111.8	18.3	Treatment Discharge
2	6-May-12	14:12	244.4	116.0	18.3	Treatment Discharge
2	6-May-12	14:13	244.5	120.3	18.1	Treatment Discharge
2	6-May-12	14:14	243.6	124.5	18.2	Treatment Discharge
2	6-May-12	14:15	244.9	128.9	18.4	Treatment Discharge
2	6-May-12	14:16	244.4	133.1	18.3	Treatment Discharge
2	6-May-12	14:17	244.4	137.4	18.3	Treatment Discharge
2	6-May-12	14:18	244.9	141.5	18.4	Treatment Discharge
2	6-May-12	14:19	243.6	145.8	18.4	Treatment Discharge
2	6-May-12	14:20	244.9	150.1	18.3	Treatment Discharge
2	6-May-12	14:21	245.1	154.4	18.1	Treatment Discharge
2	6-May-12	14:22	244.4	158.6	18.4	Treatment Discharge
2	6-May-12	14:23	244.0	162.9	18.3	Treatment Discharge
2	6-May-12	14:24	245.3	167.1	18.2	Treatment Discharge
2	6-May-12	14:25	244.0	171.4	18.0	Treatment Discharge
2	6-May-12	14:26	204.5	175.4	18.3	Treatment Discharge / Stripping
2	6-May-12	14:27	48.1	177.3	18.1	Treatment Discharge / Stripping
2	6-May-12	14:28	48.5	178.1	18.3	Treatment Discharge / Stripping
2	6-May-12	14:29	48.1	178.9	18.2	Treatment Discharge / Stripping
2	6-May-12	14:30	48.1	179.8	18.3	Treatment Discharge / Stripping
2	6-May-12	14:31	48.1	180.7	18.3	Treatment Discharge / Stripping
2	6-May-12	14:32	47.7	181.5	18.4	Treatment Discharge / Stripping
2	6-May-12	14:33	48.1	182.3	18.5	Treatment Discharge / Stripping
2	6-May-12	14:34	48.1	183.1	18.5	Treatment Discharge / Stripping
2	6-May-12	14:35	47.7	184.0	18.6	Treatment Discharge / Stripping
2	6-May-12	14:36	48.1	184.8	18.6	Treatment Discharge / Stripping
2	6-May-12	14:37	47.7	185.6	18.3	Treatment Discharge / Stripping
2	6-May-12	14:38	47.8	186.4	18.3	Treatment Discharge / Stripping
2	6-May-12	14:39	29.0	187.1	18.4	Treatment Discharge / Stripping
2	6-May-12	14:40	0.0	187.1	18.4	Finished
2	6-May-12	14:57	0.0	0.0	18.3	Finished
2	6-May-12	14:58	225.3	0.0	18.7	Recirculation
2	6-May-12	14:59	250.0	0.0	18.9	Recirculation
2	6-May-12	15:00	249.1	0.0	19.0	Recirculation
2	6-May-12	15:01	250.0	0.0	19.4	Recirculation
2	6-May-12	15:02	249.2	0.0	19.6	Recirculation
2	6-May-12	15:03	250.3	0.0	19.6	Recirculation
2	6-May-12	15:04	249.4	0.0	19.8	Recirculation
2	6-May-12	15:05	249.3	0.0	19.7	Recirculation
2	6-May-12	15:06	250.8	0.0	20.1	Recirculation
2	6-May-12	15:07	249.5	0.0	20.2	Recirculation
2	6-May-12	15:08	250.4	0.0	20.1	Recirculation
2	6-May-12	15:09	250.0	0.0	20.2	Recirculation
2	6-May-12	15:10	250.4	0.0	20.4	Recirculation
2	6-May-12	15:11	250.2	0.0	20.6	Recirculation
2	6-May-12	15:12	249.5	0.0	20.8	Recirculation
2	6-May-12	15:13	249.5	0.0	21.0	Recirculation
2	6-May-12	15:14	250.4	0.0	20.8	Recirculation
2	6-May-12	15:15	250.4	0.0	21.3	Recirculation
2	6-May-12	15:16	250.4	0.0	21.2	Recirculation
2	6-May-12	15:17	250.7	0.0	21.5	Recirculation

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
2	6-May-12	15:18	250.4	0.0	21.7	Recirculation
2	6-May-12	15:19	250.0	0.0	21.9	Recirculation
2	6-May-12	15:20	249.7	0.0	21.8	Recirculation
2	6-May-12	15:21	249.8	0.0	22.2	Recirculation
2	6-May-12	15:22	250.2	0.0	22.2	Ready to Sample
2	6-May-12	15:23	250.4	2.9	19.5	Control Discharge
2	6-May-12	15:24	250.4	7.0	18.3	Control Discharge
2	6-May-12	15:25	250.0	11.2	18.3	Control Discharge
2	6-May-12	15:26	250.1	15.3	18.3	Control Discharge
2	6-May-12	15:27	249.5	19.3	18.3	Control Discharge
2	6-May-12	15:28	249.8	23.4	18.3	Control Discharge
2	6-May-12	15:29	250.5	27.5	18.1	Control Discharge
2	6-May-12	15:30	250.6	31.6	18.1	Control Discharge
2	6-May-12	15:31	250.0	35.7	18.1	Control Discharge
2	6-May-12	15:32	249.3	39.8	18.1	Control Discharge
2	6-May-12	15:33	249.6	43.8	18.3	Control Discharge
2	6-May-12	15:34	250.3	47.9	18.2	Control Discharge
2	6-May-12	15:35	249.5	52.1	18.0	Control Discharge
2	6-May-12	15:36	250.0	56.1	18.2	Control Discharge
2	6-May-12	15:37	249.5	60.2	18.3	Control Discharge
2	6-May-12	15:38	250.4	64.3	18.1	Control Discharge
2	6-May-12	15:39	249.5	68.3	18.1	Control Discharge
2	6-May-12	15:40	248.5	72.4	18.2	Control Discharge
2	6-May-12	15:41	250.4	76.6	18.3	Control Discharge
2	6-May-12	15:42	250.1	80.7	18.1	Control Discharge
2	6-May-12	15:43	250.4	84.8	18.1	Control Discharge
2	6-May-12	15:44	249.5	88.9	18.3	Control Discharge
2	6-May-12	15:45	249.5	92.9	18.1	Control Discharge
2	6-May-12	15:46	250.0	97.0	18.3	Control Discharge
2	6-May-12	15:47	249.1	101.1	18.2	Control Discharge
2	6-May-12	15:48	250.4	105.2	18.3	Control Discharge
2	6-May-12	15:49	250.5	109.3	18.3	Control Discharge
2	6-May-12	15:50	249.9	113.4	18.3	Control Discharge
2	6-May-12	15:51	250.0	117.4	18.3	Control Discharge
2	6-May-12	15:52	250.1	121.6	18.1	Control Discharge
2	6-May-12	15:53	250.1	125.7	18.1	Control Discharge
2	6-May-12	15:54	250.3	129.7	18.1	Control Discharge
2	6-May-12	15:55	249.5	133.8	18.4	Control Discharge
2	6-May-12	15:56	249.4	137.9	18.2	Control Discharge
2	6-May-12	15:57	250.5	142.0	18.1	Control Discharge
2	6-May-12	15:58	250.0	146.1	18.0	Control Discharge
2	6-May-12	15:59	249.4	150.2	18.1	Control Discharge
2	6-May-12	16:00	250.4	154.3	18.3	Control Discharge
2	6-May-12	16:01	250.4	158.4	18.1	Control Discharge
2	6-May-12	16:02	250.0	162.5	18.1	Control Discharge
2	6-May-12	16:03	249.6	166.6	18.3	Control Discharge
2	6-May-12	16:04	250.0	170.6	18.3	Control Discharge
2	6-May-12	16:05	250.4	174.7	18.3	Control Discharge
2	6-May-12	16:06	250.1	178.8	18.3	Control Discharge
2	6-May-12	16:07	249.4	182.9	18.3	Control Discharge
2	6-May-12	16:08	250.0	187.0	18.3	Control Discharge
2	6-May-12	16:09	250.0	191.1	18.1	Control Discharge
2	6-May-12	16:10	250.0	195.2	18.3	Control Discharge
2	6-May-12	16:11	67.5	197.8	18.2	Control Discharge / Stripping

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
2	6-May-12	16:12	50.7	198.7	18.0	Control Discharge / Stripping
2	6-May-12	16:13	50.7	199.7	18.3	Control Discharge / Stripping
2	6-May-12	16:14	50.5	200.6	18.2	Control Discharge / Stripping
2	6-May-12	16:15	50.7	201.5	18.2	Control Discharge / Stripping
2	6-May-12	16:16	50.6	202.4	18.1	Control Discharge / Stripping
2	6-May-12	16:17	50.4	203.3	18.0	Control Discharge / Stripping
2	6-May-12	16:18	51.0	204.2	18.3	Control Discharge / Stripping
2	6-May-12	16:19	49.7	205.1	18.3	Control Discharge / Stripping
2	6-May-12	16:20	50.8	206.0	18.1	Control Discharge / Stripping
2	6-May-12	16:21	50.2	206.9	18.2	Control Discharge / Stripping
2	6-May-12	16:22	49.8	207.8	18.1	Control Discharge / Stripping
2	6-May-12	16:23	49.4	208.7	18.3	Control Discharge / Stripping
2	6-May-12	16:24	0.0	209.2	18.3	Finished
3	7-Oct-12	12:39	0.0	0.0	20.5	Finished
3	7-Oct-12	12:40	227.8	0.0	23.3	Sea-to-Sea
3	7-Oct-12	12:41	251.2	0.0	18.3	Sea-to-Sea
3	7-Oct-12	12:42	252.1	0.0	18.0	Sea-to-Sea
3	7-Oct-12	12:43	250.8	0.0	17.8	Sea-to-Sea
3	7-Oct-12	12:44	251.2	0.0	18.1	Sea-to-Sea
3	7-Oct-12	12:45	251.2	0.0	17.8	Sea-to-Sea
3	7-Oct-12	12:46	252.5	0.0	18.0	Sea-to-Sea
3	7-Oct-12	12:47	250.8	0.0	18.1	Sea-to-Sea
3	7-Oct-12	12:48	251.2	0.0	18.2	Sea-to-Sea
3	7-Oct-12	12:49	251.7	0.0	17.9	Sea-to-Sea
3	7-Oct-12	12:50	251.8	0.0	18.0	Sea-to-Sea
3	7-Oct-12	12:51	251.4	0.0	18.1	Sea-to-Sea
3	7-Oct-12	12:52	251.2	0.0	18.1	Sea-to-Sea
3	7-Oct-12	12:53	251.5	0.0	18.0	Ready to Sample
3	7-Oct-12	12:54	251.8	2.8	18.0	Treatment Uptake
3	7-Oct-12	12:55	251.2	6.9	18.1	Treatment Uptake
3	7-Oct-12	12:56	251.6	11.0	17.9	Treatment Uptake
3	7-Oct-12	12:57	250.5	15.1	18.0	Treatment Uptake
3	7-Oct-12	12:58	252.0	19.2	18.0	Treatment Uptake
3	7-Oct-12	12:59	253.4	23.3	18.1	Treatment Uptake
3	7-Oct-12	13:00	251.2	27.3	18.0	Treatment Uptake
3	7-Oct-12	13:01	251.7	31.4	18.2	Treatment Uptake
3	7-Oct-12	13:02	252.1	35.5	18.1	Treatment Uptake
3	7-Oct-12	13:03	251.1	39.6	18.2	Treatment Uptake
3	7-Oct-12	13:04	251.2	43.7	18.1	Treatment Uptake
3	7-Oct-12	13:05	251.0	47.8	18.0	Treatment Uptake
3	7-Oct-12	13:06	251.4	51.8	18.0	Treatment Uptake
3	7-Oct-12	13:07	252.0	55.9	18.1	Treatment Uptake
3	7-Oct-12	13:08	251.2	60.0	17.8	Treatment Uptake
3	7-Oct-12	13:09	251.5	64.1	18.1	Treatment Uptake
3	7-Oct-12	13:10	245.7	68.1	18.0	Treatment Uptake
3	7-Oct-12	13:11	252.5	72.2	18.1	Treatment Uptake
3	7-Oct-12	13:12	238.9	76.2	18.1	Treatment Uptake
3	7-Oct-12	13:13	252.1	80.2	18.0	Treatment Uptake
3	7-Oct-12	13:14	251.2	84.3	18.0	Treatment Uptake
3	7-Oct-12	13:15	237.2	88.3	17.9	Treatment Uptake
3	7-Oct-12	13:16	252.5	92.4	17.9	Treatment Uptake
3	7-Oct-12	13:17	232.9	96.3	18.1	Treatment Uptake
3	7-Oct-12	13:18	251.2	100.3	18.0	Treatment Uptake
3	7-Oct-12	13:19	252.0	104.5	18.0	Treatment Uptake

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
3	7-Oct-12	13:20	187.8	108.0	18.2	Treatment Uptake
3	7-Oct-12	13:21	238.2	112.0	18.0	Treatment Uptake
3	7-Oct-12	13:22	252.4	115.8	17.8	Treatment Uptake
3	7-Oct-12	13:23	215.1	119.7	18.2	Treatment Uptake
3	7-Oct-12	13:24	229.8	123.7	18.0	Treatment Uptake
3	7-Oct-12	13:25	252.1	127.5	17.8	Treatment Uptake
3	7-Oct-12	13:26	229.5	131.6	18.0	Treatment Uptake
3	7-Oct-12	13:27	198.0	135.0	18.0	Treatment Uptake
3	7-Oct-12	13:28	185.7	138.1	18.0	Treatment Uptake
3	7-Oct-12	13:29	182.0	141.1	18.0	Treatment Uptake
3	7-Oct-12	13:30	252.0	144.9	18.0	Treatment Uptake
3	7-Oct-12	13:31	226.6	148.9	17.8	Treatment Uptake
3	7-Oct-12	13:32	174.6	151.9	18.1	Treatment Uptake
3	7-Oct-12	13:33	250.5	155.4	17.8	Treatment Uptake
3	7-Oct-12	13:34	238.1	159.5	18.0	Treatment Uptake
3	7-Oct-12	13:35	182.3	163.0	18.1	Treatment Uptake
3	7-Oct-12	13:36	172.0	165.8	17.9	Treatment Uptake
3	7-Oct-12	13:37	251.2	169.5	18.1	Treatment Uptake
3	7-Oct-12	13:38	220.0	173.6	17.9	Treatment Uptake
3	7-Oct-12	13:39	183.1	176.8	18.1	Treatment Uptake
3	7-Oct-12	13:40	192.4	179.9	18.2	Treatment Uptake
3	7-Oct-12	13:41	184.9	183.0	18.2	Treatment Uptake
3	7-Oct-12	13:42	174.2	185.9	18.0	Treatment Uptake
3	7-Oct-12	13:43	166.5	188.6	18.0	Treatment Uptake
3	7-Oct-12	13:44	0.0	190.1	18.1	Treatment Uptake
3	7-Oct-12	13:45	0.0	190.1	17.8	Treatment Uptake
3	7-Oct-12	13:46	0.0	190.1	18.0	Treatment Uptake
3	7-Oct-12	13:47	0.0	190.1	17.9	Treatment Uptake
3	7-Oct-12	13:48	0.0	190.1	18.0	Treatment Uptake
3	7-Oct-12	13:49	153.0	190.1	18.1	Treatment Uptake
3	7-Oct-12	13:50	204.7	193.5	18.3	Treatment Uptake
3	7-Oct-12	13:51	170.8	196.6	18.1	Treatment Uptake
3	7-Oct-12	13:52	213.9	199.9	17.8	Treatment Uptake
3	7-Oct-12	13:53	209.9	203.4	17.8	Treatment Uptake
3	7-Oct-12	13:54	194.3	206.6	18.0	Treatment Uptake
3	7-Oct-12	13:55	177.3	209.5	17.9	Treatment Uptake
3	7-Oct-12	13:56	177.2	212.3	18.1	Treatment Uptake
3	7-Oct-12	13:57	173.3	215.1	18.0	Treatment Uptake
3	7-Oct-12	13:58	93.0	217.8	17.8	Treatment Uptake
3	7-Oct-12	13:59	0.0	217.8	18.0	Finished
3	7-Oct-12	14:06	0.0	0.0	17.9	Finished
3	7-Oct-12	14:07	172.9	0.0	18.1	Sea-to-Sea
3	7-Oct-12	14:08	252.0	0.0	18.1	Sea-to-Sea
3	7-Oct-12	14:09	251.4	0.0	17.8	Sea-to-Sea
3	7-Oct-12	14:10	251.2	0.0	18.0	Sea-to-Sea
3	7-Oct-12	14:11	252.1	0.0	18.0	Sea-to-Sea
3	7-Oct-12	14:12	251.5	0.0	18.0	Sea-to-Sea
3	7-Oct-12	14:13	252.0	0.0	17.8	Sea-to-Sea
3	7-Oct-12	14:14	252.1	0.0	17.9	Sea-to-Sea
3	7-Oct-12	14:15	251.2	0.0	17.7	Sea-to-Sea
3	7-Oct-12	14:16	252.1	0.8	17.8	Control Uptake
3	7-Oct-12	14:17	251.7	4.9	17.8	Control Uptake
3	7-Oct-12	14:18	252.5	9.1	18.0	Control Uptake
3	7-Oct-12	14:19	251.2	13.1	17.8	Control Uptake

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
3	7-Oct-12	14:20	251.7	17.2	17.8	Control Uptake
3	7-Oct-12	14:21	251.3	21.3	17.7	Control Uptake
3	7-Oct-12	14:22	251.8	25.3	18.0	Control Uptake
3	7-Oct-12	14:23	250.8	29.5	17.8	Control Uptake
3	7-Oct-12	14:24	251.5	33.6	17.7	Control Uptake
3	7-Oct-12	14:25	252.1	37.7	17.7	Control Uptake
3	7-Oct-12	14:26	250.4	41.7	17.8	Control Uptake
3	7-Oct-12	14:27	253.0	45.8	17.7	Control Uptake
3	7-Oct-12	14:28	250.8	49.9	18.0	Control Uptake
3	7-Oct-12	14:29	250.8	54.0	17.7	Control Uptake
3	7-Oct-12	14:30	250.0	58.1	17.8	Control Uptake
3	7-Oct-12	14:31	252.4	62.1	17.8	Control Uptake
3	7-Oct-12	14:32	252.1	66.2	17.8	Control Uptake
3	7-Oct-12	14:33	252.1	70.3	17.7	Control Uptake
3	7-Oct-12	14:34	252.0	74.5	17.7	Control Uptake
3	7-Oct-12	14:35	252.2	78.6	17.9	Control Uptake
3	7-Oct-12	14:36	252.5	82.7	18.0	Control Uptake
3	7-Oct-12	14:37	252.5	86.7	17.8	Control Uptake
3	7-Oct-12	14:38	251.2	90.8	17.8	Control Uptake
3	7-Oct-12	14:39	251.7	94.8	17.6	Control Uptake
3	7-Oct-12	14:40	253.3	99.0	17.6	Control Uptake
3	7-Oct-12	14:41	251.7	103.1	17.9	Control Uptake
3	7-Oct-12	14:42	252.1	107.2	17.8	Control Uptake
3	7-Oct-12	14:43	252.4	111.2	17.6	Control Uptake
3	7-Oct-12	14:44	252.5	115.3	17.7	Control Uptake
3	7-Oct-12	14:45	251.2	119.4	17.8	Control Uptake
3	7-Oct-12	14:46	252.5	123.5	17.8	Control Uptake
3	7-Oct-12	14:47	250.5	127.6	17.7	Control Uptake
3	7-Oct-12	14:48	252.4	131.7	17.6	Control Uptake
3	7-Oct-12	14:49	250.8	135.7	17.8	Control Uptake
3	7-Oct-12	14:50	251.5	139.9	17.6	Control Uptake
3	7-Oct-12	14:51	251.2	144.0	17.6	Control Uptake
3	7-Oct-12	14:52	251.7	148.1	17.7	Control Uptake
3	7-Oct-12	14:53	252.0	152.2	17.6	Control Uptake
3	7-Oct-12	14:54	251.2	156.2	17.6	Control Uptake
3	7-Oct-12	14:55	250.8	160.3	17.6	Control Uptake
3	7-Oct-12	14:56	251.2	164.4	17.6	Control Uptake
3	7-Oct-12	14:57	251.5	168.5	17.6	Control Uptake
3	7-Oct-12	14:58	250.9	172.6	17.7	Control Uptake
3	7-Oct-12	14:59	251.2	176.7	17.8	Control Uptake
3	7-Oct-12	15:00	252.1	180.8	17.8	Control Uptake
3	7-Oct-12	15:01	251.3	184.9	17.6	Control Uptake
3	7-Oct-12	15:02	251.2	188.9	17.8	Control Uptake
3	7-Oct-12	15:03	251.2	193.0	17.5	Control Uptake
3	7-Oct-12	15:04	251.7	197.1	17.8	Control Uptake
3	7-Oct-12	15:05	251.2	201.2	17.8	Control Uptake
3	7-Oct-12	15:06	109.4	205.1	17.8	Finished
3	12-Oct-12	11:58	0.0	0.0	20.3	Finished
3	12-Oct-12	11:59	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:00	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:01	0.0	0.0	20.3	Recirculation
3	12-Oct-12	12:02	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:03	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:04	0.0	0.0	20.5	Recirculation

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
3	12-Oct-12	12:05	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:06	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:07	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:08	0.0	0.0	20.2	Recirculation
3	12-Oct-12	12:09	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:10	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:11	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:12	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:13	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:14	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:15	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:16	0.0	0.0	20.2	Recirculation
3	12-Oct-12	12:17	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:18	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:19	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:20	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:21	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:22	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:23	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:24	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:25	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:26	0.0	0.0	20.7	Recirculation
3	12-Oct-12	12:27	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:28	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:29	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:30	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:31	0.0	0.0	20.7	Recirculation
3	12-Oct-12	12:32	0.0	0.0	20.7	Recirculation
3	12-Oct-12	12:33	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:34	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:35	0.0	0.0	20.4	Recirculation
3	12-Oct-12	12:36	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:37	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:38	0.0	0.0	20.8	Recirculation
3	12-Oct-12	12:39	0.0	0.0	20.7	Recirculation
3	12-Oct-12	12:40	0.0	0.0	20.6	Recirculation
3	12-Oct-12	12:41	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:42	0.0	0.0	20.8	Recirculation
3	12-Oct-12	12:43	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:44	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:45	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:46	0.0	0.0	20.8	Recirculation
3	12-Oct-12	12:47	0.0	0.0	20.7	Recirculation
3	12-Oct-12	12:48	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:49	0.0	0.0	19.5	Recirculation
3	12-Oct-12	12:50	0.0	0.0	20.5	Recirculation
3	12-Oct-12	12:51	223.6	0.0	24.6	Recirculation
3	12-Oct-12	12:52	250.3	0.0	24.3	Recirculation
3	12-Oct-12	12:53	239.9	0.0	24.2	Recirculation
3	12-Oct-12	12:54	241.5	0.0	24.4	Recirculation
3	12-Oct-12	12:55	241.5	0.0	20.2	Recirculation
3	12-Oct-12	12:56	240.3	0.0	21.1	Recirculation
3	12-Oct-12	12:57	241.3	0.0	21.2	Recirculation
3	12-Oct-12	12:58	240.6	0.0	20.9	Recirculation

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
3	12-Oct-12	12:59	241.5	0.0	20.9	Recirculation
3	12-Oct-12	13:00	240.6	0.0	21.3	Recirculation
3	12-Oct-12	13:01	241.5	0.0	21.1	Recirculation
3	12-Oct-12	13:02	241.4	0.0	21.4	Recirculation
3	12-Oct-12	13:03	241.2	0.0	21.4	Recirculation
3	12-Oct-12	13:04	240.3	0.0	21.8	Recirculation
3	12-Oct-12	13:05	241.7	0.0	21.9	Treatment Discharge
3	12-Oct-12	13:06	241.5	4.1	20.5	Treatment Discharge
3	12-Oct-12	13:07	242.0	8.1	19.6	Treatment Discharge
3	12-Oct-12	13:08	242.2	12.3	19.3	Treatment Discharge
3	12-Oct-12	13:09	241.9	16.3	19.7	Treatment Discharge
3	12-Oct-12	13:10	242.5	20.4	19.3	Treatment Discharge
3	12-Oct-12	13:11	241.0	24.4	19.7	Treatment Discharge
3	12-Oct-12	13:12	241.5	28.5	19.5	Treatment Discharge
3	12-Oct-12	13:13	241.5	32.7	19.2	Treatment Discharge
3	12-Oct-12	13:14	241.5	36.8	19.5	Treatment Discharge
3	12-Oct-12	13:15	241.9	40.9	19.3	Treatment Discharge
3	12-Oct-12	13:16	241.5	44.9	19.5	Treatment Discharge
3	12-Oct-12	13:17	241.5	49.0	19.4	Treatment Discharge
3	12-Oct-12	13:18	241.8	53.1	19.4	Treatment Discharge
3	12-Oct-12	13:19	241.5	57.2	19.2	Treatment Discharge
3	12-Oct-12	13:20	241.9	61.3	19.5	Treatment Discharge
3	12-Oct-12	13:21	242.0	65.3	19.5	Treatment Discharge
3	12-Oct-12	13:22	241.9	69.4	19.3	Treatment Discharge
3	12-Oct-12	13:23	241.4	73.5	19.1	Treatment Discharge
3	12-Oct-12	13:24	241.8	77.6	19.3	Treatment Discharge
3	12-Oct-12	13:25	241.9	81.7	19.3	Treatment Discharge
3	12-Oct-12	13:26	241.5	85.8	19.3	Treatment Discharge
3	12-Oct-12	13:27	241.9	89.9	19.1	Treatment Discharge
3	12-Oct-12	13:28	246.1	93.8	19.3	Treatment Discharge
3	12-Oct-12	13:29	241.9	97.9	19.3	Treatment Discharge
3	12-Oct-12	13:30	241.9	102.0	19.5	Treatment Discharge
3	12-Oct-12	13:31	241.5	106.1	19.2	Treatment Discharge
3	12-Oct-12	13:32	241.5	110.2	19.4	Treatment Discharge
3	12-Oct-12	13:33	241.9	114.2	19.2	Treatment Discharge
3	12-Oct-12	13:34	241.9	118.3	19.2	Treatment Discharge
3	12-Oct-12	13:35	241.6	122.4	19.3	Treatment Discharge
3	12-Oct-12	13:36	241.5	126.5	19.1	Treatment Discharge
3	12-Oct-12	13:37	242.0	130.6	19.3	Treatment Discharge
3	12-Oct-12	13:38	241.9	134.7	19.3	Treatment Discharge
3	12-Oct-12	13:39	242.3	138.7	19.4	Treatment Discharge
3	12-Oct-12	13:40	241.9	142.9	19.5	Treatment Discharge
3	12-Oct-12	13:41	241.5	147.0	19.5	Treatment Discharge
3	12-Oct-12	13:42	242.3	151.0	19.3	Treatment Discharge
3	12-Oct-12	13:43	241.9	155.1	19.2	Treatment Discharge
3	12-Oct-12	13:44	241.9	159.2	19.4	Treatment Discharge
3	12-Oct-12	13:45	242.3	163.3	19.5	Treatment Discharge
3	12-Oct-12	13:46	241.5	167.4	19.1	Treatment Discharge
3	12-Oct-12	13:47	76.7	170.0	19.5	Treatment Discharge / Stripping
3	12-Oct-12	13:48	48.8	170.8	19.4	Treatment Discharge / Stripping
3	12-Oct-12	13:49	48.8	171.5	19.3	Treatment Discharge / Stripping
3	12-Oct-12	13:50	48.3	172.2	19.5	Treatment Discharge / Stripping
3	12-Oct-12	13:51	48.5	173.0	19.3	Treatment Discharge / Stripping
3	12-Oct-12	13:52	48.5	173.7	19.4	Treatment Discharge / Stripping

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
3	12-Oct-12	13:53	48.1	174.4	19.3	Treatment Discharge / Stripping
3	12-Oct-12	13:54	48.1	175.1	19.3	Treatment Discharge / Stripping
3	12-Oct-12	13:55	48.1	175.9	19.6	Treatment Discharge / Stripping
3	12-Oct-12	13:56	48.1	176.6	19.6	Treatment Discharge / Stripping
3	12-Oct-12	13:57	48.1	177.3	19.5	Treatment Discharge / Stripping
3	12-Oct-12	13:58	48.0	178.1	19.5	Treatment Discharge / Stripping
3	12-Oct-12	13:59	48.2	178.8	19.5	Treatment Discharge / Stripping
3	12-Oct-12	14:00	48.6	179.5	19.4	Treatment Discharge / Stripping
3	12-Oct-12	14:01	48.1	180.2	19.3	Treatment Discharge / Stripping
3	12-Oct-12	14:02	47.7	181.0	19.3	Treatment Discharge / Stripping
3	12-Oct-12	14:03	0.0	181.2	19.3	Finished
3	12-Oct-12	14:18	17.0	0.0	19.3	Finished
3	12-Oct-12	14:19	181.0	0.0	19.2	Recirculation
3	12-Oct-12	14:20	250.4	0.0	19.5	Recirculation
3	12-Oct-12	14:21	252.2	0.0	19.8	Recirculation
3	12-Oct-12	14:22	250.4	0.0	19.7	Recirculation
3	12-Oct-12	14:23	252.5	0.0	19.9	Recirculation
3	12-Oct-12	14:24	252.1	0.0	20.5	Recirculation
3	12-Oct-12	14:25	253.0	0.0	20.3	Recirculation
3	12-Oct-12	14:26	251.5	0.0	20.7	Recirculation
3	12-Oct-12	14:27	252.5	0.0	20.8	Recirculation
3	12-Oct-12	14:28	252.1	0.0	20.9	Recirculation
3	12-Oct-12	14:29	250.8	0.0	21.1	Recirculation
3	12-Oct-12	14:30	251.7	2.5	20.3	Control Discharge
3	12-Oct-12	14:31	250.4	6.7	19.1	Control Discharge
3	12-Oct-12	14:32	252.4	10.9	19.4	Control Discharge
3	12-Oct-12	14:33	252.2	15.0	19.1	Control Discharge
3	12-Oct-12	14:34	252.5	19.2	19.4	Control Discharge
3	12-Oct-12	14:35	250.4	23.4	19.1	Control Discharge
3	12-Oct-12	14:36	252.5	27.6	19.1	Control Discharge
3	12-Oct-12	14:37	252.1	31.8	19.4	Control Discharge
3	12-Oct-12	14:38	251.7	36.0	19.4	Control Discharge
3	12-Oct-12	14:39	252.4	40.2	19.4	Control Discharge
3	12-Oct-12	14:40	252.1	44.4	19.3	Control Discharge
3	12-Oct-12	14:41	250.8	48.6	19.1	Control Discharge
3	12-Oct-12	14:42	251.2	52.8	19.3	Control Discharge
3	12-Oct-12	14:43	252.4	57.0	19.1	Control Discharge
3	12-Oct-12	14:44	251.9	61.2	19.3	Control Discharge
3	12-Oct-12	14:45	252.5	65.3	19.4	Control Discharge
3	12-Oct-12	14:46	252.1	69.6	19.1	Control Discharge
3	12-Oct-12	14:47	252.6	73.8	19.3	Control Discharge
3	12-Oct-12	14:48	250.8	77.9	19.4	Control Discharge
3	12-Oct-12	14:49	252.5	82.1	19.3	Control Discharge
3	12-Oct-12	14:50	252.5	86.2	19.1	Control Discharge
3	12-Oct-12	14:51	250.8	90.4	19.3	Control Discharge
3	12-Oct-12	14:52	252.1	94.7	19.1	Control Discharge
3	12-Oct-12	14:53	252.5	98.9	19.2	Control Discharge
3	12-Oct-12	14:54	250.8	103.1	19.1	Control Discharge
3	12-Oct-12	14:55	251.4	107.3	19.0	Control Discharge
3	12-Oct-12	14:56	252.4	111.4	19.4	Control Discharge
3	12-Oct-12	14:57	251.8	115.6	19.3	Control Discharge
3	12-Oct-12	14:58	251.2	119.8	19.1	Control Discharge
3	12-Oct-12	14:59	252.1	124.0	19.1	Control Discharge
3	12-Oct-12	15:00	251.2	128.2	19.3	Control Discharge

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
3	12-Oct-12	15:01	252.5	132.4	19.3	Control Discharge
3	12-Oct-12	15:02	252.5	136.6	19.3	Control Discharge
3	12-Oct-12	15:03	251.5	140.8	19.4	Control Discharge
3	12-Oct-12	15:04	250.9	145.0	19.1	Control Discharge
3	12-Oct-12	15:05	251.7	149.2	19.1	Control Discharge
3	12-Oct-12	15:06	251.2	153.4	19.1	Control Discharge
3	12-Oct-12	15:07	251.4	157.6	19.4	Control Discharge
3	12-Oct-12	15:08	252.1	161.8	19.3	Control Discharge
3	12-Oct-12	15:09	251.1	166.0	19.0	Control Discharge
3	12-Oct-12	15:10	251.0	170.2	19.4	Control Discharge
3	12-Oct-12	15:11	251.7	174.4	19.3	Control Discharge
3	12-Oct-12	15:12	251.2	178.6	19.3	Control Discharge
3	12-Oct-12	15:13	152.0	182.6	19.0	Control Discharge / Stripping
3	12-Oct-12	15:14	47.4	183.8	19.4	Control Discharge / Stripping
3	12-Oct-12	15:15	47.7	184.5	19.2	Control Discharge / Stripping
3	12-Oct-12	15:16	47.4	185.2	19.0	Control Discharge / Stripping
3	12-Oct-12	15:17	47.6	185.9	19.1	Control Discharge / Stripping
3	12-Oct-12	15:18	47.3	186.6	19.4	Control Discharge / Stripping
3	12-Oct-12	15:19	47.3	187.3	19.1	Control Discharge / Stripping
3	12-Oct-12	15:20	47.7	188.0	19.5	Control Discharge / Stripping
3	12-Oct-12	15:21	47.3	188.7	19.3	Control Discharge / Stripping
3	12-Oct-12	15:22	47.7	189.4	19.3	Control Discharge / Stripping
3	12-Oct-12	15:23	47.3	190.0	19.0	Control Discharge / Stripping
3	12-Oct-12	15:24	47.5	190.7	19.4	Control Discharge / Stripping
3	12-Oct-12	15:25	47.3	191.4	19.0	Control Discharge / Stripping
3	12-Oct-12	15:26	47.3	192.1	19.4	Control Discharge / Stripping
3	12-Oct-12	15:27	47.3	192.8	19.2	Control Discharge / Stripping
3	12-Oct-12	15:28	47.3	193.5	19.3	Control Discharge / Stripping
3	12-Oct-12	15:29	46.8	194.1	19.4	Control Discharge / Stripping
3	12-Oct-12	15:30	44.7	194.8	19.4	Control Discharge / Stripping
3	12-Oct-12	15:31	40.9	195.5	19.3	Control Discharge / Stripping
3	12-Oct-12	15:32	36.5	196.1	19.3	Control Discharge / Stripping
3	12-Oct-12	15:33	5.1	196.5	19.1	Control Discharge / Stripping
3	12-Oct-12	15:34	0.0	196.5	19.3	Finished
4	20-Mar-13	12:18	222.2	0.0	14.8	Finished
4	20-Mar-13	12:19	236.8	0.0	14.7	Recirculation
4	20-Mar-13	12:20	242.4	0.0	14.4	Recirculation
4	20-Mar-13	12:21	238.6	0.0	14.6	Recirculation
4	20-Mar-13	12:22	240.3	0.0	14.6	Recirculation
4	20-Mar-13	12:23	234.1	0.0	14.6	Recirculation
4	20-Mar-13	12:24	237.6	0.0	14.7	Recirculation
4	20-Mar-13	12:25	241.5	0.0	14.7	Recirculation
4	20-Mar-13	12:26	239.4	0.0	14.7	Recirculation
4	20-Mar-13	12:27	247.1	0.0	14.6	Recirculation
4	20-Mar-13	12:28	239.3	0.0	14.4	Recirculation
4	20-Mar-13	12:29	231.2	0.0	14.4	Recirculation
4	20-Mar-13	12:30	234.8	0.0	14.4	Recirculation
4	20-Mar-13	12:31	237.2	0.0	14.7	Recirculation
4	20-Mar-13	12:32	236.8	0.0	14.5	Recirculation
4	20-Mar-13	12:33	250.3	1.8	14.5	Treatment Uptake
4	20-Mar-13	12:34	250.4	5.9	14.6	Treatment Uptake
4	20-Mar-13	12:35	251.9	10.0	14.4	Treatment Uptake
4	20-Mar-13	12:36	250.8	14.1	14.3	Treatment Uptake
4	20-Mar-13	12:37	252.1	18.2	14.3	Treatment Uptake

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
4	20-Mar-13	12:38	251.1	22.3	14.4	Treatment Uptake
4	20-Mar-13	12:39	250.8	26.3	14.5	Treatment Uptake
4	20-Mar-13	12:40	249.5	30.4	14.6	Treatment Uptake
4	20-Mar-13	12:41	250.7	34.6	14.3	Treatment Uptake
4	20-Mar-13	12:42	250.8	38.7	14.6	Treatment Uptake
4	20-Mar-13	12:43	252.1	42.7	14.3	Treatment Uptake
4	20-Mar-13	12:44	254.0	46.8	14.3	Treatment Uptake
4	20-Mar-13	12:45	250.0	50.9	14.5	Treatment Uptake
4	20-Mar-13	12:46	252.1	55.0	14.3	Treatment Uptake
4	20-Mar-13	12:47	253.5	59.1	14.7	Treatment Uptake
4	20-Mar-13	12:48	250.8	63.2	14.4	Treatment Uptake
4	20-Mar-13	12:49	247.0	67.3	14.5	Treatment Uptake
4	20-Mar-13	12:50	250.6	71.3	14.4	Treatment Uptake
4	20-Mar-13	12:51	250.8	75.4	14.6	Treatment Uptake
4	20-Mar-13	12:52	251.8	79.6	14.4	Treatment Uptake
4	20-Mar-13	12:53	250.8	83.7	14.7	Treatment Uptake
4	20-Mar-13	12:54	250.9	87.8	14.7	Treatment Uptake
4	20-Mar-13	12:55	250.4	91.8	14.6	Treatment Uptake
4	20-Mar-13	12:56	250.8	95.9	14.8	Treatment Uptake
4	20-Mar-13	12:57	251.0	100.0	14.8	Treatment Uptake
4	20-Mar-13	12:58	250.7	104.1	14.6	Treatment Uptake
4	20-Mar-13	12:59	251.2	108.2	14.6	Treatment Uptake
4	20-Mar-13	13:00	250.5	112.3	14.4	Treatment Uptake
4	20-Mar-13	13:01	251.7	116.4	14.6	Treatment Uptake
4	20-Mar-13	13:02	251.1	120.4	14.5	Treatment Uptake
4	20-Mar-13	13:03	252.1	124.6	14.5	Treatment Uptake
4	20-Mar-13	13:04	247.0	128.6	14.5	Treatment Uptake
4	20-Mar-13	13:05	248.7	132.7	14.4	Treatment Uptake
4	20-Mar-13	13:06	249.1	136.8	14.8	Treatment Uptake
4	20-Mar-13	13:07	236.8	140.8	14.4	Treatment Uptake
4	20-Mar-13	13:08	250.4	144.9	14.6	Treatment Uptake
4	20-Mar-13	13:09	250.8	149.0	14.7	Treatment Uptake
4	20-Mar-13	13:10	250.7	153.1	14.6	Treatment Uptake
4	20-Mar-13	13:11	250.4	157.2	14.8	Treatment Uptake
4	20-Mar-13	13:12	250.8	161.2	14.6	Treatment Uptake
4	20-Mar-13	13:13	251.0	165.3	14.7	Treatment Uptake
4	20-Mar-13	13:14	245.3	169.3	14.4	Treatment Uptake
4	20-Mar-13	13:15	249.0	173.2	14.7	Treatment Uptake
4	20-Mar-13	13:16	249.1	177.3	14.6	Treatment Uptake
4	20-Mar-13	13:17	232.5	181.4	14.4	Treatment Uptake
4	20-Mar-13	13:18	242.2	185.2	14.4	Treatment Uptake
4	20-Mar-13	13:19	260.6	189.2	14.6	Treatment Uptake
4	20-Mar-13	13:20	252.1	193.2	14.6	Treatment Uptake
4	20-Mar-13	13:21	250.1	197.3	14.6	Treatment Uptake
4	20-Mar-13	13:22	251.0	201.4	14.8	Treatment Uptake
4	20-Mar-13	13:23	250.8	205.5	14.5	Treatment Uptake
4	20-Mar-13	13:24	251.2	209.6	14.8	Treatment Uptake
4	20-Mar-13	13:25	0.0	212.7	14.6	Finished
4	20-Mar-13	13:29	156.1	212.7	14.6	Finished
4	20-Mar-13	13:30	253.4	212.7	14.6	Sea-to-Sea
4	20-Mar-13	13:31	252.5	212.7	14.8	Sea-to-Sea
4	20-Mar-13	13:32	252.5	212.7	14.8	Sea-to-Sea
4	20-Mar-13	13:33	252.5	212.7	14.4	Sea-to-Sea
4	20-Mar-13	13:34	252.1	213.4	14.7	Control Uptake

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
4	20-Mar-13	13:35	253.7	217.5	14.6	Control Uptake
4	20-Mar-13	13:36	253.4	221.6	14.4	Control Uptake
4	20-Mar-13	13:37	253.4	225.7	14.4	Control Uptake
4	20-Mar-13	13:38	252.5	229.7	14.2	Control Uptake
4	20-Mar-13	13:39	253.8	233.8	14.4	Control Uptake
4	20-Mar-13	13:40	253.5	237.9	14.1	Control Uptake
4	20-Mar-13	13:41	262.7	242.1	14.4	Control Uptake
4	20-Mar-13	13:42	262.7	246.4	14.4	Control Uptake
4	20-Mar-13	13:43	264.5	250.7	14.6	Control Uptake
4	20-Mar-13	13:44	263.6	255.0	14.6	Control Uptake
4	20-Mar-13	13:45	263.6	259.3	14.1	Control Uptake
4	20-Mar-13	13:46	263.2	263.7	14.4	Control Uptake
4	20-Mar-13	13:47	263.2	268.0	14.2	Control Uptake
4	20-Mar-13	13:48	262.6	272.3	14.2	Control Uptake
4	20-Mar-13	13:49	263.2	276.6	13.8	Control Uptake
4	20-Mar-13	13:50	263.2	280.9	14.0	Control Uptake
4	20-Mar-13	13:51	263.2	285.2	13.9	Control Uptake
4	20-Mar-13	13:52	262.9	289.6	13.6	Control Uptake
4	20-Mar-13	13:53	262.7	293.9	14.1	Control Uptake
4	20-Mar-13	13:54	264.0	298.2	14.2	Control Uptake
4	20-Mar-13	13:55	264.0	302.5	14.1	Control Uptake
4	20-Mar-13	13:56	262.5	306.8	14.2	Control Uptake
4	20-Mar-13	13:57	263.7	311.2	13.8	Control Uptake
4	20-Mar-13	13:58	262.7	315.5	13.8	Control Uptake
4	20-Mar-13	13:59	263.3	319.8	14.4	Control Uptake
4	20-Mar-13	14:00	263.2	324.1	13.9	Control Uptake
4	20-Mar-13	14:01	262.9	328.4	14.2	Control Uptake
4	20-Mar-13	14:02	263.2	332.7	14.6	Control Uptake
4	20-Mar-13	14:03	262.3	337.1	14.7	Control Uptake
4	20-Mar-13	14:04	263.9	341.4	14.6	Control Uptake
4	20-Mar-13	14:05	263.0	345.7	14.2	Control Uptake
4	20-Mar-13	14:06	262.6	350.0	14.1	Control Uptake
4	20-Mar-13	14:07	263.1	354.3	14.6	Control Uptake
4	20-Mar-13	14:08	263.6	358.6	14.6	Control Uptake
4	20-Mar-13	14:09	262.4	362.9	14.6	Control Uptake
4	20-Mar-13	14:10	262.8	367.3	14.4	Control Uptake
4	20-Mar-13	14:11	262.7	371.6	14.3	Control Uptake
4	20-Mar-13	14:12	262.9	375.9	14.4	Control Uptake
4	20-Mar-13	14:13	263.2	380.3	14.4	Control Uptake
4	20-Mar-13	14:14	263.6	384.5	14.3	Control Uptake
4	20-Mar-13	14:15	262.7	388.8	14.4	Control Uptake
4	20-Mar-13	14:16	263.2	393.2	14.8	Control Uptake
4	20-Mar-13	14:17	263.2	397.5	14.8	Control Uptake
4	20-Mar-13	14:18	263.2	401.8	14.6	Control Uptake
4	20-Mar-13	14:19	262.7	406.2	14.8	Control Uptake
4	20-Mar-13	14:20	263.5	410.4	14.9	Control Uptake
4	20-Mar-13	14:21	262.5	414.7	15.0	Control Uptake
4	20-Mar-13	14:22	262.7	419.0	14.8	Control Uptake
4	20-Mar-13	14:23	262.5	423.4	15.0	Control Uptake
4	20-Mar-13	14:24	263.2	427.7	14.8	Control Uptake
4	20-Mar-13	14:25	0.0	431.3	14.8	Finished
4	25-Mar-13	12:48	17.3	0.0	15.9	Finished
4	25-Mar-13	12:49	122.7	0.0	19.5	Recirculation
4	25-Mar-13	12:50	277.3	0.0	19.0	Recirculation

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
4	25-Mar-13	12:51	215.0	0.0	18.3	Recirculation
4	25-Mar-13	12:52	241.8	0.0	16.6	Recirculation
4	25-Mar-13	12:53	245.2	0.0	16.6	Recirculation
4	25-Mar-13	12:54	255.5	0.0	16.8	Recirculation
4	25-Mar-13	12:55	255.5	0.0	16.8	Recirculation
4	25-Mar-13	12:56	250.8	0.0	17.2	Recirculation
4	25-Mar-13	12:57	257.2	0.0	16.9	Recirculation
4	25-Mar-13	12:58	253.2	2.2	17.2	Treatment Discharge
4	25-Mar-13	12:59	250.0	6.6	16.2	Treatment Discharge
4	25-Mar-13	13:00	252.2	10.9	15.5	Treatment Discharge
4	25-Mar-13	13:01	253.3	15.2	15.4	Treatment Discharge
4	25-Mar-13	13:02	253.7	19.6	15.7	Treatment Discharge
4	25-Mar-13	13:03	253.2	23.9	15.6	Treatment Discharge
4	25-Mar-13	13:04	250.4	28.3	15.6	Treatment Discharge
4	25-Mar-13	13:05	251.2	32.6	15.5	Treatment Discharge
4	25-Mar-13	13:06	252.5	37.0	15.9	Treatment Discharge
4	25-Mar-13	13:07	252.5	41.4	15.6	Treatment Discharge
4	25-Mar-13	13:08	253.8	45.7	15.7	Treatment Discharge
4	25-Mar-13	13:09	252.5	50.0	15.4	Treatment Discharge
4	25-Mar-13	13:10	253.0	54.3	15.7	Treatment Discharge
4	25-Mar-13	13:11	253.3	58.7	15.6	Treatment Discharge
4	25-Mar-13	13:12	252.5	63.0	15.3	Treatment Discharge
4	25-Mar-13	13:13	251.0	67.4	15.8	Treatment Discharge
4	25-Mar-13	13:14	252.8	71.7	15.8	Treatment Discharge
4	25-Mar-13	13:15	251.1	76.1	15.8	Treatment Discharge
4	25-Mar-13	13:16	253.8	80.4	15.8	Treatment Discharge
4	25-Mar-13	13:17	253.0	84.8	15.4	Treatment Discharge
4	25-Mar-13	13:18	250.4	89.2	15.7	Treatment Discharge
4	25-Mar-13	13:19	253.7	93.5	15.4	Treatment Discharge
4	25-Mar-13	13:20	252.1	97.8	15.7	Treatment Discharge
4	25-Mar-13	13:21	252.0	102.2	15.8	Treatment Discharge
4	25-Mar-13	13:22	252.5	106.5	15.4	Treatment Discharge
4	25-Mar-13	13:23	253.0	110.9	15.6	Treatment Discharge
4	25-Mar-13	13:24	252.2	115.3	15.6	Treatment Discharge
4	25-Mar-13	13:25	253.7	119.6	15.8	Treatment Discharge
4	25-Mar-13	13:26	250.4	123.9	15.4	Treatment Discharge
4	25-Mar-13	13:27	250.8	128.2	15.8	Treatment Discharge
4	25-Mar-13	13:28	250.4	132.6	15.6	Treatment Discharge
4	25-Mar-13	13:29	252.1	137.0	15.4	Treatment Discharge
4	25-Mar-13	13:30	253.8	141.4	15.7	Treatment Discharge
4	25-Mar-13	13:31	253.4	145.7	15.5	Treatment Discharge
4	25-Mar-13	13:32	253.8	150.0	15.7	Treatment Discharge
4	25-Mar-13	13:33	251.3	154.3	15.6	Treatment Discharge
4	25-Mar-13	13:34	252.1	158.6	15.6	Treatment Discharge
4	25-Mar-13	13:35	251.2	163.1	15.4	Treatment Discharge
4	25-Mar-13	13:36	253.8	167.4	15.7	Treatment Discharge
4	25-Mar-13	13:37	251.0	171.7	15.7	Treatment Discharge
4	25-Mar-13	13:38	219.3	176.1	15.4	Treatment Discharge
4	25-Mar-13	13:39	54.9	177.6	15.8	Treatment Discharge / Stripping
4	25-Mar-13	13:40	48.4	178.5	15.5	Treatment Discharge / Stripping
4	25-Mar-13	13:41	49.8	179.4	15.6	Treatment Discharge / Stripping
4	25-Mar-13	13:42	50.2	180.3	15.8	Treatment Discharge / Stripping
4	25-Mar-13	13:43	49.8	181.2	16.0	Treatment Discharge / Stripping
4	25-Mar-13	13:44	50.1	182.1	16.0	Treatment Discharge / Stripping

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
4	25-Mar-13	13:45	49.8	183.0	15.8	Treatment Discharge / Stripping
4	25-Mar-13	13:46	49.8	183.9	16.0	Treatment Discharge / Stripping
4	25-Mar-13	13:47	49.6	184.8	16.0	Treatment Discharge / Stripping
4	25-Mar-13	13:48	50.2	185.8	15.5	Treatment Discharge / Stripping
4	25-Mar-13	13:49	49.4	186.7	15.8	Treatment Discharge / Stripping
4	25-Mar-13	13:50	49.4	187.6	15.6	Treatment Discharge / Stripping
4	25-Mar-13	13:51	40.4	188.3	16.0	Treatment Discharge / Stripping
4	25-Mar-13	13:52	33.3	188.9	16.0	Treatment Discharge / Stripping
4	25-Mar-13	13:53	0.0	188.9	15.6	Treatment Discharge / Stripping
4	25-Mar-13	13:54	0.0	188.9	15.8	Finished
4	25-Mar-13	14:06	230.4	0.0	15.6	Finished
4	25-Mar-13	14:07	250.8	0.0	15.6	Recirculation
4	25-Mar-13	14:08	253.8	0.0	15.4	Recirculation
4	25-Mar-13	14:09	252.5	0.0	15.9	Recirculation
4	25-Mar-13	14:10	252.6	0.0	15.6	Recirculation
4	25-Mar-13	14:11	252.5	0.0	15.8	Recirculation
4	25-Mar-13	14:12	253.5	0.0	16.0	Recirculation
4	25-Mar-13	14:13	252.1	0.0	16.0	Recirculation
4	25-Mar-13	14:14	253.1	0.0	16.6	Recirculation
4	25-Mar-13	14:15	252.5	0.0	16.6	Recirculation
4	25-Mar-13	14:16	252.5	0.0	16.4	Recirculation
4	25-Mar-13	14:17	252.5	0.0	17.1	Recirculation
4	25-Mar-13	14:18	253.0	0.0	16.7	Recirculation
4	25-Mar-13	14:19	253.5	0.0	17.1	Ready to Sample
4	25-Mar-13	14:20	252.5	3.6	16.2	Control Discharge
4	25-Mar-13	14:21	253.7	7.9	15.4	Control Discharge
4	25-Mar-13	14:22	253.0	12.2	15.4	Control Discharge
4	25-Mar-13	14:23	253.4	16.4	15.8	Control Discharge
4	25-Mar-13	14:24	252.9	20.7	15.6	Control Discharge
4	25-Mar-13	14:25	253.0	25.1	15.4	Control Discharge
4	25-Mar-13	14:26	253.4	29.4	15.7	Control Discharge
4	25-Mar-13	14:27	252.5	33.8	15.5	Control Discharge
4	25-Mar-13	14:28	272.1	38.1	15.5	Control Discharge
4	25-Mar-13	14:29	252.5	42.4	15.3	Control Discharge
4	25-Mar-13	14:30	252.9	46.7	15.7	Control Discharge
4	25-Mar-13	14:31	252.8	51.1	15.4	Control Discharge
4	25-Mar-13	14:32	252.5	55.4	15.3	Control Discharge
4	25-Mar-13	14:33	252.1	59.7	15.8	Control Discharge
4	25-Mar-13	14:34	252.5	64.0	15.3	Control Discharge
4	25-Mar-13	14:35	252.5	68.2	15.8	Control Discharge
4	25-Mar-13	14:36	252.5	72.6	15.3	Control Discharge
4	25-Mar-13	14:37	253.0	76.9	15.7	Control Discharge
4	25-Mar-13	14:38	252.5	81.3	15.5	Control Discharge
4	25-Mar-13	14:39	252.1	85.6	15.6	Control Discharge
4	25-Mar-13	14:40	252.5	89.9	15.8	Control Discharge
4	25-Mar-13	14:41	252.6	94.2	15.5	Control Discharge
4	25-Mar-13	14:42	253.0	98.5	15.3	Control Discharge
4	25-Mar-13	14:43	252.4	102.8	15.7	Control Discharge
4	25-Mar-13	14:44	252.6	107.1	15.8	Control Discharge
4	25-Mar-13	14:45	252.5	111.5	15.7	Control Discharge
4	25-Mar-13	14:46	252.5	115.8	15.5	Control Discharge
4	25-Mar-13	14:47	252.5	120.1	15.3	Control Discharge
4	25-Mar-13	14:48	252.5	124.4	15.7	Control Discharge
4	25-Mar-13	14:49	253.0	128.7	15.3	Control Discharge

Golden Bear Facility Automation System Output
Trojan Ballast Water Management System
IMO G8 Shipboard Testing

Test	Date (dd-mm-yy)	Time (PST)	Ballast Water Flow Rate (m ³ /hr)	Total Ballast Water Volume (m ³)	Temperature (°C)	Sampling Status
4	25-Mar-13	14:50	252.5	133.0	15.2	Control Discharge
4	25-Mar-13	14:51	253.3	137.3	15.5	Control Discharge
4	25-Mar-13	14:52	252.6	141.7	15.4	Control Discharge
4	25-Mar-13	14:53	252.7	146.0	15.3	Control Discharge
4	25-Mar-13	14:54	252.2	150.3	15.4	Control Discharge
4	25-Mar-13	14:55	252.5	154.6	15.8	Control Discharge
4	25-Mar-13	14:56	252.5	158.9	15.3	Control Discharge
4	25-Mar-13	14:57	252.1	163.2	15.7	Control Discharge
4	25-Mar-13	14:58	252.1	167.6	15.7	Control Discharge
4	25-Mar-13	14:59	252.6	171.9	15.7	Control Discharge
4	25-Mar-13	15:00	253.4	176.2	15.5	Control Discharge
4	25-Mar-13	15:01	252.6	180.5	15.8	Control Discharge
4	25-Mar-13	15:02	253.3	184.8	15.3	Control Discharge
4	25-Mar-13	15:03	253.0	189.2	15.7	Control Discharge
4	25-Mar-13	15:04	252.5	193.5	15.5	Control Discharge
4	25-Mar-13	15:05	252.1	197.7	15.3	Control Discharge
4	25-Mar-13	15:06	79.2	201.4	15.3	Control Discharge / Stripping
4	25-Mar-13	15:07	48.5	202.3	15.2	Control Discharge / Stripping
4	25-Mar-13	15:08	49.4	203.2	15.6	Control Discharge / Stripping
4	25-Mar-13	15:09	49.7	204.1	15.2	Control Discharge / Stripping
4	25-Mar-13	15:10	49.4	205.0	15.6	Control Discharge / Stripping
4	25-Mar-13	15:11	49.7	205.9	15.4	Control Discharge / Stripping
4	25-Mar-13	15:12	49.4	206.8	15.5	Control Discharge / Stripping
4	25-Mar-13	15:13	49.4	207.7	15.5	Control Discharge / Stripping
4	25-Mar-13	15:14	49.5	208.7	15.7	Control Discharge / Stripping
4	25-Mar-13	15:15	47.3	209.5	15.8	Control Discharge / Stripping
4	25-Mar-13	15:16	43.9	210.1	15.8	Control Discharge / Stripping
4	25-Mar-13	15:17	33.2	210.8	15.7	Control Discharge / Stripping
4	25-Mar-13	15:18	0.0	210.8	15.8	Finished

Appendix G Data Quality Indicators

Golden Bear Facility Data Quality Indicators

Trojan Ballast Water Management System

IMO G8 Shipboard Testing

Table 1 – Engineering Data Quality Indicators

Indicator	Analytical Method	Data Quality Indicator Target C.V. (%)	Test Event	C.V. (%)
Ballast Water Flow, Rosemount 8711 Flow Meter				
Representativeness	Device / System Characteristic	≥90%	Entire Test	100%
Accuracy	Factory Calibration	±3%	Entire Test	±0.25%
Precision	Device / System Characteristic	0.1	Entire Test	0.1
Bias	n/a	n/a	n/a	n/a
Comparability	% Difference: Total Flow vs. Branch Flow	≤10%	Event 255, Uptake	1.37%
			Event 260, Treatment Discharge	0.96%
			Event 261, Control Discharge	1.01%
			Event 309, Uptake	0.65%
			Event 310, Treatment Discharge	0.57%
			Event 311, Control Discharge	0.96%
			Event 353, Treatment Uptake	2.14%
			Event 354, Control Uptake	0.03%
			Event 356, Treatment Discharge	1.75%
			Event 357, Control Discharge	0.69%
			Event 426, Uptake	0.69%
			Event 428, Treatment Discharge	1.55%
			Event 429, Control Discharge	1.40%
Completeness	% Complete: Parameter Log Duration to Event Duration	≥90%	Event 255, Uptake	100%
			Event 260, Treatment Discharge	100%
			Event 261, Control Discharge	100%
			Event 309, Uptake	100%
			Event 310, Treatment Discharge	100%
			Event 311, Control Discharge	100%
			Event 353, Treatment Uptake	100%
			Event 354, Control Uptake	100%
			Event 356, Treatment Discharge	100%
			Event 357, Control Discharge	100%
			Event 426, Uptake	100%
			Event 428, Treatment Discharge	100%
			Event 429, Control Discharge	100%

Indicator	Analytical Method	Data Quality Indicator Target C.V. (%)	Test Event	C.V. (%)
Ballast Water Volume, TMS LevelCom 100 Tank Level Indicator				
Representativeness	Device / System Characteristic	≥90%	All Tests	100%
Accuracy	Factory Calibration	±5%	All Tests	±0.2%
Precision	Device / System Characteristic	0.1	All Tests	0.1
Bias	n/a	n/a	n/a	n/a
Comparability	% Difference: Tank Volume vs. Flow Totalizer	≤10%	Test 1, Uptake	0.61%
			Test 1, Treatment Discharge	4.10%
			Test 1, Control Discharge	2.40%
			Test 2, Uptake	3.92%
			Test 2, Treatment Discharge	3.50%
			Test 2, Control Discharge	7.81%
			Test 3, Treatment Uptake	8.85%
			Test 3, Control Uptake	1.41%
			Test 3, Treatment Discharge	0.67%
			Test 3, Control Discharge	2.59%
			Test 4, Uptake	4.36%
			Test 4, Treatment Discharge	3.58%
			Test 4, Control Discharge	3.52%
Completeness	% Complete: Parameter Log Duration to Event Duration	≥90%	Test 1, Uptake	100%
			Test 1, Treatment Discharge	100%
			Test 1, Control Discharge	100%
			Test 2, Uptake	100%
			Test 2, Treatment Discharge	100%
			Test 2, Control Discharge	100%
			Test 3, Treatment Uptake	100%
			Test 3, Control Uptake	100%
			Test 3, Treatment Discharge	100%
			Test 3, Control Discharge	100%
			Test 4, Uptake	100%
			Test 4, Treatment Discharge	100%
			Test 4, Control Discharge	100%
Sample Water Flow, Seametrics SPX				
Representativeness	Device / System Characteristic	≥90%	All Tests	100%
Accuracy	Factory Calibration	±5%	All Tests	±1%
Precision	Device / System Characteristic	0.3	All Tests	0.1
Bias	n/a	n/a	n/a	n/a
Comparability	% Difference: Totalizer Volume vs. Graduated Volume	≤10%	All Tests - Sample Tub A1	1.33%*
			All Tests - Sample Tub A2	2.65%
			All Tests - Sample Tub A3	2.94%

Indicator	Analytical Method	Data Quality Indicator Target C.V. (%)	Test Event	C.V. (%)
Completeness	% Complete: Parameter Log Duration to Event Duration	≥90%	All Tests - Sample Tub B1	3.57%
			All Tests - Sample Tub B2	3.29%
			All Tests - Sample Tub B3	4.99%
			Test 1, Uptake	100%
			Test 1, Treatment Discharge	100%
			Test 1, Control Discharge	100%
			Test 2, Uptake	100%
			Test 2, Treatment Discharge	100%
			Test 2, Control Discharge	100%
			Test 3, Treatment Uptake	100%
			Test 3, Control Uptake	100%
			Test 3, Treatment Discharge	100%
			Test 3, Control Discharge	100%
			Test 4, Uptake	100%
			Test 4, Treatment Discharge	100%
			Test 4, Control Discharge	100%

*Data outlier identified for this indicator, excluded from DQI analysis.

n/a = not analyzed

Table 2 – Biological Data Quality Indicators

Parameter	Analytical Method	Data Quality Indicator* Target C.V. (%)	Control Sample C.V. (%)	Treated Sample C.V. (%)	Notes
≥50 µm (organisms/m ³)	Visual determination of live status (30x magnification)	<31%	15%	53%	
<50 µm & ≥10 µm, MPN (organisms/mL)	MPN determination of cultivable phytoplankton; fluorometric determination of growth	<60%	64%	3%	
<50 µm & ≥10 µm, Flow Cytometry (organisms/mL)	Flow cytometric determination of live cells tagged with fluorescein diacetate (FDA)	<42%	20%	96%	
Indicator Microbes, <i>E. coli</i> (CFU/100 mL)	MPN determination IDEXX proprietary, enzyme-based MPN kit (Colilert™)	<64%	96%	0%	<i>E. coli</i> not observed in any treated samples
Indicator Microbes, <i>Enterococci</i> (CFU/100 mL)	MPN determination IDEXX proprietary, enzyme-based MPN kit (Enterolert™)	<42%	23%	139%	

Parameter	Analytical Method	Data Quality Indicator* Target C.V. (%)	Control Sample C.V. (%)	Treated Sample C.V. (%)	Notes
Indicator Microbes, <i>Vibrio Cholerae</i> (CFU/100 mL)	Antibody detection; New Horizons Diagnostics, Cholera Smart™ II (Type 01) and Bengal Smart™ II (Type 0139)	Not applicable	0%	0%	No observation of <i>V. cholera</i> >1 CFU/100 mL has been found in any sample analyzed at GBF
Indicator Microbes, Heterotrophic Plate Counts (CFU/mL)	Total colony forming units (CFU) on agar substrate	<90%	37%	49%	

* Data quality indicator is estimated as twice the empirically measured coefficient of variation for sample replicates analyzed by GBF for the given assay. C.V. = coefficient of variation; n/a = not analyzed

Table 3 – Water Quality Data Quality Indicators

Parameter	Analytical Method	Data Quality Indicators* Target C.V. (%)	Control Samples C.V. (%)	Treated Samples C.V. (%)	Notes
pH	Beckman Model 70 pH meter with ThermoOrion Ross combination electrode	±0.1 accuracy	±0.013 pH units	±0.010 pH units	Based on re-analysis of pH standards.
Total Suspended Solids (mg/L)	Gravimetric weight determination (Mettler balance)	<11%	6.2%	5.0%	
Particulate Organic Carbon (mg/L)	CHN combustion analysis	<33%	8.8%	6.4%	
Dissolved Organic Carbon (mg/L)	High temperature catalytic oxidation (McC Campbell Analytical, Inc., Pittsburg, CA; EPA approved laboratory)	± 0.1 mg/L (cited by McC Campbell Analytical)	0.04 mg/L	n/a	All reports from McC Campbell state: <i>All analyses were completed satisfactorily and all QC samples were found to be within our control limits.</i>
Chlorophyll <i>a</i> (µg/L)	Solvent extraction, fluorometric analysis	<13%	12%	12%	

* Data quality indicator is estimated as twice the empirically measured coefficient of variation for sample replicates analyzed by GBF for the given assay. C.V. = coefficient of variation; n/a = not analyzed

Appendix H Organism Composition and Taxonomy

Golden Bear Facility Organism Composition and Taxonomy

Trojan Ballast Water Management System

IMO G8 Shipboard Testing

Table 1 - ≥50 µm Organism Composition

Test	Taxonomy												
	<i>Calanoida</i> (adult & nauplii)	<i>Harpacticoida</i> (adult & nauplii)	<i>Tintinnida</i> (adult)	<i>Rotifera</i> (adult)	<i>Bivalvia</i> (veliger)	<i>Gastropoda</i> (veliger)	<i>Cirripedia</i> (nauplii)	<i>Polychaeta</i> (larva)	<i>Amphipoda</i> (adult)	<i>Bryozoa</i> (cyphonautes)	<i>Decapoda</i> (zoeae)	<i>Nematoda</i>	Ichthyoplankton
Test 1	x	x	x	x	x	x	x	x		x	x	x	
Test 2	x	x	x	x	x		x				x		x
Test 3	x	x	x	x	x	x	x	x					
Test 4	x	x	x	x	x	x	x	x	x	x	x		

Table 2 - ≥10 µm but <50 µm Organism Composition

Test	Taxonomy																
	<i>Asterionella</i>	<i>Ceratium divaricatum</i>	<i>Ceratium lineatum</i>	<i>Chaetoceros</i>	<i>Corethron</i>	<i>Cochlodinium</i>	<i>Cymbella</i>	<i>Ditylum</i>	<i>Dinophysis</i>	<i>Gonyaulax</i>	<i>Lauderia</i>	<i>Navicula</i>	<i>Odontella</i>	<i>Oxyphysis</i>	<i>Pleurosigma</i>	<i>Rhizosolenia</i>	<i>Skeletonema</i>
Test 1						x	x	x			x	x	x		x		x
Test 2	x			x	x			x					x			x	x
Test 3		x	x						x	x			x	x			
Test 4	x						x						x	x	x		x

Table 3 - ≥10 µm but <50 µm Phytoplankton Composition, MPN Results

Test	Taxonomy
Test 1	<i>Thalassiosira</i> , <i>Chaetoceros</i> , <i>Stephanopyxis</i> , flagellates, and <10 µm chain forming diatom
Test 2	<i>Thalassiosira</i> , <i>Chaetoceros</i> , flagellates, and <10 µm chain forming diatom
Test 3	NA
Test 4	<i>Thalassiosira</i> , <i>Chaetoceros</i> , <i>Odontella</i> , flagellates, and aggregates of <10µm organisms